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(21) International Application Number: PCT/CA99/00619 (22) International Filing Date: 8 July 1999 (08.07.99) (30) Priority Data: 09/111,895 8 July 1998 (08.07.98) US (71) Applicant (for all designated States except US): ANORMED INC. [CA/CA]; No. 200, 20353 – 64th Avenue, Surrey, British Columbia V2Y 1N5 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): BRIDGER, Gary, James [GB/US]; 445 S. Garden Street, Bellingham, WA 98225 (US). BOEHRINGER, Eva, Maria [CA/CA]; 404 – 1558 Fir Street, White Rock, British Columbia V4B 4B7 (CA). WANG, Zhongren [CA/CA]; #218, 5380 – 208 Street, Langley, British Columbia V3A 2C2 (CA). SCHOLS, Dominique [BE/BE]; Kiersveld 9, B-3020 Herent (BE). SKERLJ, Renato, Tony [NZ/US]; 9062 Shearwater Road, Blaine, WA 98230 (US). BOGUCKI, David, Earl [CA/CA]; 203, 16071 – 82nd Avenue, Surrey, British Columbia V3S 2L6 (CA).	(74) Agents: ROBINSON, J., Chistopher et al.; Fetherstonhaugh & Co., Suite 2200, 650 West Georgia Street, Box 11560, Vancouver, British Columbia V6B 4N8 (CA). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: ANTIVIRAL MACROCYCLIC COMPOUNDS (57) Abstract <p>The present invention is drawn to novel antiviral compounds, pharmaceutical compositions and their use. More specifically this invention is drawn to derivatives of monocyclic polyamines which have activity in standard tests against HIV- or FIV-infected cells as well as other biological activity related to binding of ligands to chemokine receptors that mediate a number of mammalian embryonic developmental processes.</p>		

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ANTIVIRAL MACROCYCLIC COMPOUNDS

The present invention is drawn to novel antiviral compounds, pharmaceutical compositions and their use. More specifically this invention is drawn to derivatives of monocyclic polyamines which have activity in standard tests against HIV-infected cells as well as other biological activity related to binding of ligands to chemokine receptors that mediate a number of mammalian embryonic developmental processes. The present invention further includes methods of treating various diseases mediated by chemokine receptor-ligand binding.

BACKGROUND OF THE INVENTION

Approximately 40 human chemokines have been described, that function, at least in part, by modulating a complex and overlapping set of biological activities important for the movement of lymphoid cells and extravasation and tissue infiltration of leukocytes in response to inciting agents (See, for example: P. Ponath, *Exp. Opin. Invest. Drugs*, 7:1-18, 1998). These chemotactic cytokines, or chemokines, constitute a family of proteins, approximately 8-10 kDa in size. Chemokines appear to share a common structural motif, that consists of 4 conserved cysteines involved in maintaining tertiary structure. There are two major subfamilies of chemokines: the "CC" or β -chemokines and the "CXC" or α -chemokines. The receptors of these chemokines are classified based upon the chemokine that constitutes the receptor's natural ligand. Receptors of the β -chemokines are designated "CCR"; while those of the α -chemokines are designated "CXCR".

Chemokines are considered to be principal mediators in the initiation and maintenance of inflammation. More specifically, chemokines have been found to play an important role in the regulation of endothelial cell function, including proliferation, migration and differentiation during angiogenesis and re-endothelialization after injury (Gupta *et al.*, *J. Biolog. Chem.*, 7:4282-4287, 1998). Two specific chemokines have been implicated in the etiology of infection by human immunodeficiency virus (HIV).

In most instances, HIV initially binds via its gp120 envelope protein to the CD4 receptor of the target cell. A conformational change appears to take place in the gp120 which results in

its subsequent binding to a chemokine receptor, such as CCR-5 (Wyatt *et al.*, *Science*, 280:1884-1888 (1998)). HIV-1 isolates arising subsequently in the infection bind to the CXCR-4 chemokine receptor. In view of the fact that the feline immunodeficiency virus, another related retrovirus, binds to a chemokine receptor without needing to bind first to the CD4 receptor, suggests that chemokine receptors may be the primordial obligate receptors for immunodeficiency retroviruses (Richardson *et al.*, *J. Virol.* 73:661 (1999)).

Following the initial binding by HIV to CD4, virus-cell fusion results, which is mediated by members of the chemokine receptor family, with different members serving as fusion cofactors for macrophage-tropic (M-tropic) and T cell line-tropic (T-tropic) isolates of HIV-1 (Carroll *et al.*, *Science*, 276: 273-276 1997). During the course of infection within a patient, it appears that a majority of HIV particles shift from the M-tropic to the more aggressive T-tropic viral phenotype (Miedema *et al.*, *Immune. Rev.*, 140:35 (1994)). Curiously, the M-tropic viral phenotype correlates with the virus's ability to enter the cell following binding of the CCR-5 receptor, while the T-tropic viral phenotype correlates with viral entry into the cell following binding and membrane fusion with the CXCR-4 receptor. Clinically observations suggest that patients who possess genetic mutations in the CCR-5 appear resistant or less susceptible to HIV infection.

However, the binding of chemokine receptors to their natural ligands appears to serve a more evolutionary and central role than only as mediators of HIV infection. The chemokine receptor, CXCR-4 has been found to be essential for the vascularization of the gastrointestinal tract (Tachibana *et al.*, *Nature*, 393:591-594 (1998)) as well as haematopoiesis and cerebellar development (Zou *et al.*, *Nature*, 393:591-594 (1998)). Interference with any of these important functions served by the binding of pre-B-cell growth-stimulating factor/stromal derived factor (PBSF/SDF-1) to the CXCR-4 chemokine receptor results in lethal deficiencies in vascular development, haematopoiesis and cardiogenesis. Similarly, fetal cerebellar development appears to rely upon the effective functioning of CXCR-4 in neuronal cell migration and patterning in the central nervous system. This G-protein-coupled chemokine receptor appears to play a critical role in ensuring the necessary patterns of migration of granule cells in the cerebellar anlage. Interactions of SDF-1 and CXCR4 are also important in maintaining B-cell lineage and in retaining stem cells in bone marrow (Peled *et al.*, *Science* 283: 845 (1999); Springer *et al.*, *Immunity* 10:463 (1999))

In attempting to better understand the relationship between chemokines and their receptors, recent experiments to block the binding of HIV to the CXCR-4 chemokine receptor were carried out through the use of monoclonal antibodies or small molecules that appear to suggest a useful therapeutic strategy (Schols *et al.*, *J. Exp. Med.* 186:1383-1388 (1997); Schols
5 *et al.*, *Antiviral Research* 35:147-156 (1997)). Small molecules, such as bicyclams, appear to specifically interfere with the CXCR-4 binding and not CCR-5 binding (Donzella *et al.*, *Nature Medicine*, 4:72-77 (1998)). These experiments demonstrated interference with HIV entry and membrane fusion into the target cell *in vitro*. Additional experiments monitoring the calcium flux or Ca^{2+} mobilization assay demonstrated that a bicyclam also functioned as an antagonist to
10 signal transduction resulting from the binding of stromal derived factor or SDF-1 α , the natural chemokine to CXCR-4.

Further, the etiology or association of chemokine receptor binding in the proliferation of glioblastoma tumor cells has been reported by Sehgal *et al.*, *J. of Surg. Oncolo.* 69:99-104 (1998) ("Sehgal I") and Sehgal *et al.*, *J. of Surg. Oncolo.* 69:239-248 (1998) ("Sehgal II"). The
15 role of CXCR4 of its binding to its receptor appears to play a significant role in the formation and/or proliferation of glioblastoma cells. The inhibition of the binding by CXCR4 to its natural receptor ligand by compounds of the present invention, such as AMD 3100, offer a new drug in the treatment tumors of central nervous system that are mediated or associated with chemokines, such as CXCR4.

20 Additionally, CXC chemokines have been found to regulate or are associated with the regulation of angiogenesis in non-small cell lung cancer (see: Arenberg, *et al.*, *J. of Leukocyte Biol.*; 62:554-562 (1997); and Moore *et al.* *TCM*, vol 8(2): 51-58 (1998) Elsevier Science, Inc.). The role of CXC chemokines and the binding to their respective receptors appear to play a significant role in the formation and/or proliferation of non-small cell lung cancer. The
25 inhibition of the binding of these CXC chemokines to their natural receptor ligands by compounds of the present invention, such as AMD 3100, offer a new drug in the treatment tumors such as non-small cell lung cancer that are mediated or associated with increased levels of chemokines.

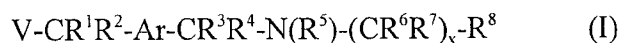
U.S. Pat. No. 5,583,131, U.S. Pat. No. 5,698,546 and U.S. Pat. No. 5,817,807 disclose
30 cyclic compounds that are active against HIV-1 and HIV-2 in *in vitro* tests. We have now discovered that these compounds exhibit anti-HIV (anti-human immunodeficiency virus) and

anti-FIV (anti-feline immunodeficiency virus) activities due to their binding to the chemokine receptor 4 (CXCR-4 or Fusin receptor), expressed on the surface of certain cells of the immune system (Este *et al.*, *Mol. Pharmacol.* 55:67 (1999); Egberink *et al.*, *J. Virol in press* (1999)). This competitive binding thereby protects these target cells from infection by HIV which utilize the CXCR-4 receptor for entry. We have discovered that the disclosed compounds also antagonize the binding, signaling and chemotactic effects of the natural CXC-chemokine for CXCR-4, stromal cell-derived factor 1 α (SDF-1 α). Herein, we further disclose novel compounds that demonstrate protective effects against HIV infection of target cells by inhibition of binding *in vitro* to the CC-5 receptor (CCR-5).

SUMMARY OF THE INVENTION

The present invention provides novel compounds, that demonstrate protective effects on target cells from HIV infection as well as demonstrate other biological activities related to the ability of these compounds to inhibit the binding by the natural ligand to its chemokine receptor.

Accordingly, the present invention provides a macrocyclic compound of formula I:



wherein V is a cyclic polyamine moiety having a total of 9 to 24 members and from 2 to 6, but preferably from 3 to 6, optionally substituted amino nitrogens optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

R¹ to R⁷ may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C₁₋₆ alkyl;

R⁸ is a heterocyclic group, a substituted aromatic group, or a mercaptan group;

Ar is an aromatic or heteroaromatic ring each optionally substituted at single or multiple positions with electrons-donating or withdrawing groups;

x is 1 or 2;

and the acid addition salts and metal complexes thereof.

Preferably V is a 14- to 17 membered fused or unfused ring system, such as a cyclam

system or a 4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene system or a derivative thereof, and especially a cyclam system or derivative thereof. The moiety V may be substituted at C or N non-linking atoms, suitably by hydroxyl, alkoxy, thiol, thioalkyl or any other atom or group which does not adversely affect the activity or toxicity of the compounds but may reduce the basicity of the amines, for example halogen, nitro, carboxy, carboxyamido, sulphonic acid or phosphate. Suitably the fused aromatic or heteroaromatic ring is phenyl, pyridine, pyrimidine, pyrazine, imidazole or thiazole. Preferably, the fused aromatic or heteroaromatic ring is phenyl or pyridine.

10 Preferably R¹ to R⁷ are each hydrogen.

Preferably R⁸ is selected from pyridine, pyrimidine, pyrazine, imidazole, thiophene, thiophenyl, aminobenzyl, piperidinyl, piperazinyl or a mercaptan group.

15 Preferably Ar is phenyl. Preferred substituents are alkyl, aryl, amino, alkoxy, hydroxy, halogen, carboxyl and carboxamido.

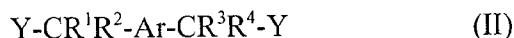
The invention also includes what may be termed as "pro-drug", that is protected forms of the compounds, which release the compound after administration to a patient. For example, the compound may carry a protective groups which is split off by hydrolysis in body fluids e.g. in the bloodstream, thus releasing active compound or are oxidized or reduced in body fluids to release the compound. A discussion on pro-drugs may be found in "Smith and Williams' Introduction to the Principles of Drug Design", H.J. Smith, Wright, Second Edition, London 1988.

25 Acid addition salts, for example hydrochlorides, and non-toxic labile metal complexes of compounds of formula I are also active compounds according to the present invention. Non-toxic in the present tense has to be considered with reference to the prognosis for the infected patient without treatment. Copper and zinc complexes are preferred although other metals such as nickel may be considered, whereas less labile metals such as cobalt and rhodium are less preferred because of likely lower selectivity.

30 Compounds of formula I are novel. Accordingly, a further aspect of the invention

provides a process for the preparation of a compound of formula I which comprises the following steps:

- (i) nucleophilic attack by the cyclic polyamine V having a single unprotected amine nitrogen, all other amine nitrogen atoms being protected, on an excess of a compound of formula II

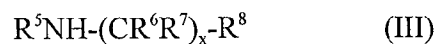


wherein R^1 to R^4 and Ar are as hereinbefore defined, and each Y is an active substituent which can be displaced by the unprotected nitrogen of polyamine V and is preferably selected from Br, Cl, I, methane sulphonate, 4-toluenesulphonate, trifluoromethane sulphonate.

It is well within the capabilities and knowledge of the skilled synthetic chemist to protect the amine nitrogens of cyclic polyamines, and it is preferred to use substitution by methanesulphonyl and/or toluenesulphonyl and/or diethoxyphosphoryl (see: Bridger *et al*, *J. Med. Chem.*, 38:366-378 (1995); Bridger *et al*, U.S. Pat. 5,583,131 or Bridger *et al*, U.S. Pat. 5,698,546) and/or nitrobenzenesulfonyl (Fukuyama *et al.*, *Tetrahedron Letters* 1995, 36, 6373-6374).

The protected polyamine V is firstly reacted with a 5-to 10-fold excess of a compound of formula II in a solvent such as acetonitrile or dimethylformamide, tetrahydrofuran or dioxane and in the presence of a base, for example sodium carbonate or potassium carbonate. The reaction generally proceeds at room temperature to elevated temperature to give a cyclic polyamine in which all amine nitrogens are protected. In general, a mixture of products will be obtained and we have found that the product can conveniently be purified by silica gel chromatography or crystallization.

Nucleophilic attack of a compound of formula III

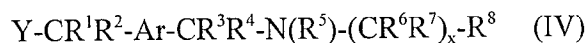


wherein R^5 to R^8 and x are as hereinbefore defined on the product of the reaction

described at (I) above, and subsequently de-protecting the amine nitrogens. The reaction with an excess of a compound of formula III is carried out under similar conditions to the reaction with the polyamine V.

The de-protection step is suitably carried out by re-fluxing the protected molecule in a mixture of aqueous HBr and acetic acid or concentrated sulphuric acid, or in the case of diethoxyphosphoryl in the presence of gaseous hydrogen chloride or gaseous hydrogen bromide in acetic acid; in the case of nitrobenzenesulfonyl deprotection, a mercaptan such as thiophenol or mercaptoacetic acid in the presence of a suitable base such as potassium carbonate, cesium carbonate, sodium hydroxide or lithium hydroxide in a solvent such as dimethylformamide, acetonitrile, tetrahydrofuran or dioxane is used. This reaction generally proceeds at room temperature to elevated temperatures to give a polyamine in which the nitrogens are deprotected. Alternatively, and accordingly, a further aspect of the invention provides a process for the preparation of compounds of Formula I which comprises the following steps:

(i) nucleophilic attack by the cyclic polyamine V having a single unprotected amine nitrogen, all other amine nitrogens being protected, with an excess of a compound of formula (IV)

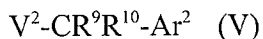


Wherein R^1 to R^4 and x , R^6 to R^8 and Ar are as hereinbefore defined and Y is an active substituent which can be displaced by the unprotected nitrogen of cyclic polyamine V as hereinbefore defined. In this case, the intended substituent R^5 is hydrogen but for convenience, the nitrogen is protected as a nitrobenzenesulfonyl or diethoxyphosphoryl group.

The protected polyamine V is first reacted with a compound of formula IV using similar conditions to the reactions with compounds of formula II and formula III as described above and the product of this reaction is subjected to deprotection of the amine nitrogens on the polyamine and at R^5 .

The deprotection steps were carried out as described above. For convenience, a sequential combination of these deprotection reactions may be used when a mixture of any of: methanesulphonyl; toluenesulphonyl; diethoxyphosphoryl; or nitrobenzenesulfonyl groups are present.

The novel compounds further comprise a macrocyclic compound of general formula V:



where V^2 is a cyclic polyamine moiety having a total of 9 to 24 members and from 2 to 6, but preferably from 3 to 6, optionally substituted amine nitrogens spaced by two or more
5 optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring; where R^9 and R^{10} may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C_{1-6} alkyl; further, where Ar^2 is an aromatic, fused aromatic, heterocyclic or fused heterocyclic ring each optionally substituted at single or multiple positions with electron-donating or withdrawing groups and/or
10 aromatic and heterocyclic groups and their alkyl derivatives thereof; and the acid addition salts and metal complexes.

These novel compounds have demonstrated anti-HIV activity in an *in vitro* screen assay as presented in Table 1. These novel compounds have also demonstrated biological activity in inhibiting CXCR-4 specific monoclonal antibody (12G5) from binding to CXCR-4 on SUP-T1
15 cells by AMD compounds. These data are shown in Table 2 for AMD3100 (1,1'-[1,4-phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane), AMD3465 (N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine) and six new compounds: AMD 7049; AMD 7050; AMD 7051; AMD 7058; AMD 7059; and AMD 7063.

20 Data showing inhibition of the increased Ca^{2+} flux induced by SDF-1 in SUP-T1 cells (inhibition of signal transduction) by AMD compounds are shown in Table 3 for AMD3100, AMD3465 and compounds: AMD 7049; AMD 7050; AMD 7051; AMD 7058; AMD 7059; and AMD 7063.

Several novel compounds also inhibited infection of the cell line U87.CD4.CCR5 by the
25 M-tropic HIV-1 strain BaL, which exclusively utilizes the CCR-5 co-receptor for entry. These data are shown in Table 4.

The experimental procedures for the mAb binding assay, the inhibition of Ca^{2+} flux, and inhibition of infection by the HIV-1 BaL strain in U87.CD4.CCR5 cells would be readily understood by the skilled artisan. For example, see: Schols *et al.*, *J. Exp. Med.* 186:1383-1388
30 (1997); Schols *et al.*, *Antiviral Research* 35:147-156 (1997); and Donzella *et al.*, *Nature*

Medicine, 4:72-77 (1998). Also, the characterization of the CXCR-4 specific monoclonal antibody 12G5 is taught by Hoxie *et al.*, *Cell*, 87:745-756 (1996).

Citation of the above documents is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the date or representation as to the contents
5 of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents. Further, all documents referred to throughout this application are incorporated in their entirety by reference herein.

Having now generally described the invention, the same will be more readily understood
10 through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

As mentioned above, the compounds of the invention have activity against viral infections, especially retrovirus infections and specifically HIV. Accordingly, a further aspect of the invention provides a compound of formula I or formula V for use in medicine. More
15 specifically, there is provided the use of a compound of formula I or formula V in the manufacture of a medicament for the treatment of HIV-infected patients. In the alternative, there is provided a method of treating an HIV-infected patient comprising administering to said patient, a pharmaceutically effective amount of a compound of formula I or formula V. Although compounds of formula I or formula V could be administered as the raw material, it is
20 preferable to present them in the form of a pharmaceutical composition comprising a compound of formula I or formula V as active ingredient in admixture with a pharmaceutically acceptable diluent or carrier and optionally one or more other therapeutic ingredients, such compositions providing a further aspect of the invention.

In all aspects of the invention, it is understood that meso forms, enantiomers and
25 resolved optically active forms of the compounds of formula I or formula V are also included. Also, it is to be considered within the invention, compounds of formula I or formula V diluted with non-toxic or other active substances.

BRIEF DESCRIPTION OF THE DRAWINGS

30 Fig. 1, shows the structural formula of compound AMD 3465.

Fig. 2, shows the structural formula of compound AMD 3538.

- Fig. 3; shows the structural formula of compound AMD 3500.
- Fig. 4, shows the structural formula of compound AMD 3499.
- Fig. 5; shows the structural formula of compound AMD 3498.
- Fig. 6; shows the structural formula of compound AMD 3497.
- 5 Fig. 7; shows the structural formula of compound AMD 3516.
- Fig. 8; shows the structural formula of compound AMD 3530.
- Fig. 9; shows the structural formula of compound AMD 3517.
- Fig. 10; shows the structural formula of compound AMD 3544.
- Fig. 11; shows the structural formula of compound AMD 3543.
- 10 Fig. 12; shows the structural formula of compound AMD 3529.
- Fig. 13; shows the structural formula of compound AMD 7049.
- Fig. 14; shows the structural formula of compound AMD 7050.
- Fig. 15; shows the structural formula of compound AMD 7051.
- Fig. 16; shows the structural formula of compound AMD 7059.
- 15 Fig. 17; shows the structural formula of compound AMD 7063.
- Fig. 18; shows the structural formula of compound AMD 7058.
- Fig. 19; shows the structural formula of compound AMD 7032.
- Fig. 20; shows the structural formula of compound AMD 7048.
- Fig. 21; shows the structural formula of compound AMD 7060.
- 20 Fig. 22; shows the structural formula of compound AMD 7061.
- Fig. 23; shows the structural formula of compound AMD 3451.
- Fig. 24; shows the structural formula of compound AMD 3454.
- Fig. 25; shows the structural formula of compound AMD 3472.
- Fig. 26; shows the structural formula of compound AMD 3526.
- 25 Fig. 27; shows the structural formula of compound AMD 3100.
- Fig. 28; shows the structural formula of compound AMD 3484.
- Fig. 29, shows the cumulative incidence of collagen-induced arthritis in laboratory animals following treatment or immunization with compound AMD 3100.
- Fig. 30, shows the change in body weight of laboratory animals following treatment
- 30 with compound AMD 3100.
- Fig. 31, shows the structural formula of compound AMD 8630.

Fig. 32, shows the structural formula of compound AMD 7097.

Fig. 33, shows the structural formula of compound AMD 8631.

Fig. 34, shows the structural formula of compound AMD-Exp 1.

Fig. 35, shows the structural formula of compound AMD 7450.

5 Fig. 36, shows the structural formula of compound AMD-Exp 2.

Fig. 37, shows the structural formula of compound AMD 7463.

Fig. 38, shows the structural formula of compound AMD-Exp 3.

DETAILED DESCRIPTION OF THE INVENTION

10 Terms as used herein are based upon their art recognized meaning unless otherwise indicated and should be clearly understood by the ordinary skilled artisan. The present invention will now be illustrated by the following preparative Examples.

General Procedure A

15 1-[1-Methylene-4-(bromomethylene)phenylene]-4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane

To a stirred solution of 4,8,11-Tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane (See Bridger *et al. J Med. Chem.* 1995, 38, 366-378) (6.1g, 0.01mol) and K₂CO₃ (1.89g, 0.013mol) in CH₃CN (150ml) was added α,α' -dibromo-p-xylene (13.2g, 0.05mol) and the reaction mixture stirred at 70°C for 1 hour. The solution was cooled to room temperature and the solvent removed under reduced pressure. The residue was partitioned between brine (50ml) and CH₂Cl₂ (100ml). The organic phase was separated, dried (Na₂SO₄) and concentrated to a minimum volume. The solid was filtered off and the solvent evaporated under reduced pressure to give the crude product as a pale yellow oil. Purification by column chromatography in silica gel (CH₂Cl₂/CH₃OH, 25:1) gave 1-[1-methylene-4-(bromomethylene)phenylene]-4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane (4.7g, 59%) as a pale yellow oil. ¹H NMR (CDCl₃) δ 1.21-1.37 (m, 18H), 1.66-1.74 (m, 2H), 1.82-1.91 (m, 2H), 2.30-2.35 (m, 2H), 2.58-2.63 (m, 2H), 2.99-3.16 (m, 12H), 3.48 (s, 2H), 3.95-4.07 (m, 12H), 4.48 (s, 2H), 7.21-7.35 (4H).

General Procedure B

Second alkylation of the bromobenzyl cyclam intermediate with an amine (see for example:
Bridger *et al. J. Med. Chem.* 1995, 38, 366-378)

To a solution of the appropriate amine (5.0 equiv.) in dry CH₃CN (5mL) containing a
5 suspension of K₂CO₃ (1.5 equiv.) at 80°C was added dropwise with stirring a solution of 1-[1-
methylene-4-(bromomethylene)phenylene]-4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-
tetraazacyclotetradecane (0.6mmol) in CH₃CN (10ml) over 15-20 min. After stirring for a
further 1 hour at 80°C the solution was concentrated to dryness and the residue was partitioned
between CH₂Cl₂ and water. The organic layer was separated and washed with water (3x) then
10 dried (MgSO₄) and evaporated. The crude residue was purified by column chromatography on
silica gel eluting with 5-15% MeOH/CH₂Cl₂ to afford a viscous oil.

General Procedure C

De-protection of the diethoxyphosphoramidate groups using HBr/HOAc at room temperature
15 (see for example: Bridger *et al. J. Med. Chem.* 1995, 38, 366-378)

To a stirred solution of the protected cyclam derivative from procedure B (0.1-0.5mmol)
in acetic acid (3mL) was added 30% HBr in acetic acid (Aldrich, 5mL) and the solution was
stirred at room temperature for 14 hours. The resulting precipitate was collected by filtration
and washed with acetic acid then Et₂O. The solid was then dissolved in H₂O (3mL) and treated
20 with charcoal (100mg) and the mixture was heated to 80°C for 30min. The hot solution was
filtered through celite and the filtrate was concentrated to approximately 1mL after which acetic
acid was added resulting in the immediate formation of a white precipitate. The white solid was
collected by filtration and dried *in vacuo*.

The following compounds were prepared by these methods:

25

Example 1

N-[1,4,8,11-Tetraazacyclotetradecanyl]-1,4-phenylenebis(methylene)]-2-(amino-methyl)pyridine
hexahydrobromide (AMD 3465)

White solid: Mp 200-205°C (dec); ¹H NMR (D₂O) δ 2.04 (m, 4H), 3.20-3.40 (m, 8H),
30 3.40-3.60 (m, 8H), 4.34 (s, 2H), 4.38 (s, 2H), 4.51 (s, 2H), 7.50 (m, 4H), 7.75 (t, 1H, *J*=6.6 Hz),
7.82 (d, 1H, *J*=7.9 Hz), 8.26 (t, 1H, *J*=7.9 Hz), 8.63 (d, 1H, *J*=5.3 Hz); ¹³C NMR (D₂O) δ 18.30,

18.96, 37.04, 37.28, 37.40, 40.92, 41.13, 41.49, 44.26, 47.61, 48.01, 51.29, 58.88, 127.46, 127.75, 130.40, 131.05, 131.23, 131.47, 132.10, 132.44, 144.95, 145.81, 146.01; FAB MS m/z 493 ($M+H^{81}Br$, 7), 491 ($M+H^{79}Br$, 7), 411 ($M+H$, 100).

Anal. ($C_{24}H_{38}N_6 \cdot 6HBr$); Calc. C, 32.36; H, 4.98; N, 9.44; Br, 53.21. Found C, 32.20; H, 5.00; N, 9.30; Br, 53.10.

Example 2

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-*N*-methyl-2-(aminomethyl)pyridine hexahydrobromide hydrate (AMD 3538)

White solid: Mp 220-225°C (dec); 1H NMR (D_2O) δ 2.06 (m, 4H), 2.76 (s, 3H), 3.20-3.65 (m, 16H), 4.47 (bs, 4H), 4.65 (s, 2H), 7.54 (bs, 4H), 7.80 (t, 1H), 7.87 (d, 1H), 8.28 (t, 1H), 8.68 (d, 1H); ^{13}C NMR (D_2O) δ 18.14, 18.75, 18.89, 36.74, 37.04, 37.15, 37.62, 40.38, 40.72, 40.91, 41.28, 44.05, 47.50, 56.98, 58.88, 60.28, 127.60, 128.86, 130.78, 130.96, 132.16, 132.64, 144.91, 145.04, 146.12; FAB MS m/z 507 ($M+H^{81}Br$, 27), 507 ($M+H^{79}Br$, 22), 425 ($M+H$, 100).

Anal. ($C_{25}H_{40}N_6 \cdot 6HBr \cdot 1.5H_2O$); Calc. C, 32.04; H, 5.27; N, 8.97; Br, 51.16. Found C, 31.88; H, 5.30; N, 8.93; Br, 51.00.

Example 3

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-amino-methylpyridine hexahydrobromide (AMD 3500)

White solid: mp 201-204°C (dec); 1H NMR (D_2O) δ 1.91-2.12 (m, 4H), 3.00-3.49 (m, 16H), 4.13 (s, 2H), 4.34 (s, 2H), 4.53 (s, 2H), 7.39-7.57 (m, 4H), 8.02 (d, 2H, $J=6.3$ Hz), 8.74 (d, 2H, $J=6.3$ Hz); ^{13}C NMR (D_2O) δ 18.26, 18.88, 36.94, 37.29, 37.36, 40.89, 41.06, 41.44, 44.21, 47.61, 49.17, 51.43, 59.02, 127.84, 130.21, 131.64, 132.15, 132.45, 142.19, 151.67; FAB MS m/z 493 ($M+H^{81}Br$, 8), 491 ($M+H^{79}Br$, 10), 411 ($M+H$, 83), 320 (37), 247 (58), 201 (100).

Anal. ($C_{24}H_{38}N_6 \cdot 6HBr$); Calc. C, 32.17; H, 4.95; N, 9.34; Br, 53.50. Found C, 32.16; H, 5.03; N, 9.41; Br, 53.28.

Example 4*N*-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-3-(amino-methyl)pyridine
hexahydrobromide (AMD 3499)

White solid: mp 198-202°C (dec); ¹H NMR (D₂O) δ 1.83-2.07 (m, 4H), 2.96-3.47 (m, 16H), 4.11 (s, 2H), 4.32 (s, 2H), 4.49 (s, 2H), 7.38-7.56 (m, 4H), 8.04 (t, 1H, *J*=6.4 Hz), 8.63 (d, 1H, *J*=8.3 Hz), 8.76 (d, 1H, *J*=5.6 Hz), 8.86 (s, 1H); ¹³C NMR (D₂O) δ 18.23, 18.87, 36.92, 37.29 (2C), 40.88, 41.05, 41.43, 44.17, 47.22, 47.60, 51.18, 59.04, 128.29, 130.01, 131.49, 132.14, 132.66 (2C), 142.55, 142.76, 148.98; FAB MS *m/z* 493 (M+H⁸¹Br, 7), 491 (M+H⁷⁹Br, 6), 411 (M+H, 100), 320 (33), 247 (24).

Anal. (C₂₄H₃₈N₆·6HBr); Calc. C, 32.17; H, 4.95; N, 9.34; Br, 53.50. Found C, 32.08; H, 5.02; N, 9.25; Br, 53.28.

Example 5*N*-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-(2-amino-methyl-5-methyl)pyrazine pentahydrobromide (AMD 3498)

White solid: mp 194-197°C (dec); ¹H NMR (D₂O) δ 1.93-2.12 (m, 4H), 2.42 (s, 3H), 3.25 (s, 8H), 3.48 (s, 8H), 4.28 (s, 2H), 4.30 (s, 2H), 4.33 (s, 2H), 7.44 (s, 4H), 8.33 (s, 1H), 8.46 (s, 1H); ¹³C NMR (D₂O) δ 18.01, 18.72, 19.80, 36.66, 37.05, 37.13, 40.70, 40.89, 41.27, 43.99, 47.47, 48.14, 50.61, 59.06, 129.97, 131.43, 132.04, 132.99, 140.93, 144.98, 146.49, 153.51; FAB MS *m/z* 509 (M+H⁸¹Br, 17), 507 (M+H⁷⁹Br, 15), 426 (M+H, 100), 320 (21), 247 (20).

Anal. (C₂₄H₃₉N₇·5.5HBr); Calc. C, 33.10; H, 5.15; N, 11.26; Br, 50.47. Found C, 32.80; H, 5.41; N, 11.00; Br, 50.58.

Example 6*N*-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-ethyl)pyridine
hexahydrobromide (AMD 3497)

White solid: mp 195-198°C (dec); ¹H NMR (D₂O) δ 1.98-2.17 (m, 4H), 3.20-3.38 (m, 8H), 3.38-3.63 (m, 12H), 4.27 (s, 2H), 4.39 (s, 2H), 7.50 (s, 4H), 7.80-7.89 (m, 2H), 8.42 (m, 1H), 8.58 (d, 1H, *J*=5.8 Hz); ¹³C NMR (D₂O) δ 18.51, 19.14, 29.85, 37.56 (3C), 41.21, 41.41,

41.82, 44.57, 45.27, 47.83, 51.10, 58.74, 126.35, 127.93, 130.66, 131.27, 131.99, 132.69, 141.89, 147.79, 150.91; FAB MS m/z 507 ($M+H^{81}Br$, 40), 505 ($M+H^{79}Br$, 34), 425 ($M+H$, 100).

Anal. ($C_{25}H_{40}N_6 \cdot 6HBr$); Calc. C, 32.99; H, 5.09; N, 9.23; Br, 52.67. Found C, 32.79; H, 5.34; N, 9.11; Br, 52.45.

5

Example 7

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-methyl)thiophene pentahydrobromide (AMD 3516)

White solid: mp 245-248°C (dec); 1H NMR (D_2O) δ 1.87-2.12 (m, 4H), 3.02-3.51 (m, 16H), 4.17 (s, 4H), 4.38 (s, 2H), 6.97 (t, 1H, $J=3.9$ Hz), 7.13 (d, 1H, $J=3.1$ Hz), 7.41 (s, 5H); ^{13}C NMR (D_2O) δ 18.80, 19.52, 38.03, (3C), 41.59 (2C), 42.21, 44.89 (2C), 48.15, 49.83, 58.52, 128.13, 129.12, 131.15, 131.47, 131.50, 131.90, 132.42, 132.87; FAB MS m/z 498 ($M+H^{81}Br$, 11), 496 ($M+H^{79}Br$, 9), 416 ($M+H$, 53), 218 (100), 201 (64).

Anal. ($C_{23}H_{37}N_5S \cdot 5HBr$); Calc. C, 33.68; H, 5.16; N, 8.54; Br, 48.71. Found C, 33.85; H, 5.22; N, 8.50; Br, 48.52.

15

Example 8

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-ethyl)mercaptan pentahydrobromide dihydrate (AMD 3530)

White solid: mp 234-236°C (dec); 1H NMR (D_2O) δ 1.75-2.05 (m, 4H), 2.75-3.45 (m, 20H), 4.05 (s, 2H), 4.15 (s, 2H), 7.35 (s, 4H); FAB MS m/z 462 ($M+H^{81}Br$, 15), 460 ($M+H^{79}Br$, 15), 380 ($M+H$, 100), 300 (64), 279 (47), 239 (49).

Anal. ($C_{20}H_{37}N_5S \cdot 5HBr \cdot 2H_2O \cdot 0.5HOAc$) requires C, 29.67; H, 5.69; N, 8.24; Br, 46.99. Found C, 29.31; H, 5.72; N, 8.25; Br, 46.64.

25

Example 9

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-amino-benzylamine pentahydrobromide (AMD 3517)

White solid: mp 203-206°C (dec); 1H NMR (D_2O) δ 1.85-2.13 (m, 4H), 3.02-3.58 (m, 16H), 4.23 (s, 2H), 4.31 (s, 4H), 7.23-7.54 (m, 8H); ^{13}C NMR (D_2O) δ 18.03, 19.29, 37.78 (3C), 41.37 (2C), 42.00, 44.82, 46.25, 47.96, 51.16, 58.68, 124.04, 124.40, 129.40, 130.75, 131.21

30

(2C), 131.88, 131.96, 132.46, 132.83; FAB MS m/z 507 ($M+H^{81}Br$, 15), 505 ($M+H^{79}Br$, 18), 425 ($M+H$, 100), 320 (30), 201 (51).

Anal. ($C_{25}H_{40}N_6 \cdot 5.75HBr \cdot 0.5H_2O$). Calc. C, 33.42; H, 5.19; N, 9.35; Br, 51.14. Found C, 33.69; H, 5.35; N, 9.00; Br, 51.13.

5

Example 10

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-amino-benzylamine
hexahydrobromide (AMD 3544)

Yellow solid: mp 120-125°C (dec); 1H NMR (D_2O) δ 1.8-2.0 (m, 4H), 2.9-3.4 (m, 10 16H), 4.1 (s, 2H), 4.18 (s, 4H), 7.2-7.5 (m, 8H); ^{13}C NMR (D_2O) δ 18.86, 19.57, 38.14, 41.76, 43.74, 45.14, 48.24, 50.14, 50.42, 51.49, 58.38, 124.13, 131.13, 131.30, 131.83, 131.92, 131.96, 132.67; FAB MS m/z 507 ($M+H^{81}Br$, 5), 505 ($M+H^{79}Br$, 5), 425 ($M+H$, 45), 201 (47), 155 (75), 106 (100).

Anal. ($C_{25}H_{40}N_6 \cdot 6HBr \cdot HOAc$) requires C, 33.43; H, 5.19; N, 8.66; Br, 49.42; O, 3.30. 15 Found C, 33.42; H, 5.49; N, 8.62; Br, 49.23.

Example 11

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-(amino-ethyl)imidazole
hexahydrobromide (AMD 3543)

Off white solid: mp 135-140°C (dec); 1H NMR (D_2O) δ 1.75 (m, 2H), 1.90 (m, 2H), 20 2.70-3.27 (m, 20H), 3.77 (s, 2H), 4.14 (s, 2H), 7.18 (s, 1H), 7.25 (d, 2H, $J=7.97$ Hz), 7.37 (d, 2H, $J=7.97$ Hz), 8.48 (s, 1H); FAB MS m/z 496 ($M+H^{81}Br$, 5), 494 ($M+H^{79}Br$, 5), 414 ($M+H$, 17), 201 (15).

Anal. ($C_{23}H_{39}N_7 \cdot 6HBr$) requires C, 30.73; H, 5.04; N, 10.91; Br, 53.32. Found C, 30.39; 25 H, 5.41; N, 10.41; Br, 53.66.

Example 12

N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-benzylamine
pentahydrobromide (AMD 3529)

Off white solid: mp 245-250°C (dec); 1H NMR (D_2O) δ 1.9-2.1 (m, 4H), 3.2-3.6 (m, 30 16H), 4.12 (s, 2H), 4.15 (s, 2H), 4.36 (s, 2H), 7.30 (s, 5H), 7.41 (d, 2H, $J=8.3$ Hz) 7.46 (d, 2H,

$J=8.3$ Hz); ^{13}C NMR (D_2O) δ 18.43, 19.06, 37.29, 37.46, 37.63, 41.09, 41.32, 41.68, 44.46, 47.74, 50.18, 51.00, 58.79, 129.53, 129.97, 130.18, 130.35, 130.68, 131.18, 131.92, 133.14; FAB MS m/z 492 ($\text{M}+\text{H}^{81}\text{Br}$, 13), 490 ($\text{M}+\text{H}^{79}\text{Br}$, 13), 410 ($\text{M}+\text{H}$, 100), 201 (36).

Anal. ($\text{C}_{25}\text{H}_{39}\text{N}_5\cdot 5\text{HBr}$); requires C, 36.88; H, 5.45; N, 8.60; Br, 49.07. Found C, 36.79; H, 5.56; N, 8.48; Br, 48.79.

The compounds of the invention were tested in a screen by the MTT method (*J. Virol. Methods* 120:309-321 (1988)). MT-4 cells ($2.5 \times 10^4/\text{well}$) were challenged with HIV-1 (HTLV-III_B) or HIV-2 (LAV-2 ROD) at a concentration of 100 CCID₅₀ and incubated in the presence of various concentrations of the test compounds, which were added immediately after challenge with the virus. After 5 days culture at 37°C in a CO₂ incubator, the number of viable cells was assessed by the MTT (tetrazolium) method. Antiviral activity and cytotoxicity of the compounds are expressed in Table 1 below as EC₅₀ ($\mu\text{g}/\text{ml}$) and CC₅₀ ($\mu\text{g}/\text{ml}$), respectively. The potential therapeutic usefulness was assessed by calculating a Selectivity Index (SI) corresponding to the ratio of CC₅₀ to EC₅₀.

Table 1				
Anti-HIV activity data				
Compound	CC ₅₀ ($\mu\text{g}/\text{mL}$)	EC ₅₀ ($\mu\text{g}/\text{mL}$)		SI
		HIV-1 (IIIB)	HIV-2	HIV-1
1 AMD3465	>250	0.008	0.032	3×10^4
2 AMD3538	209	0.1	6.7	2.0×10^3
3 AMD3500	>250	0.6	10.3	417
4 AMD3499	>250	1.8	28.5	138
5 AMD3498	>250	0.2	7.1	1.2×10^3
6 AMD3497	>250	1.8	3.8	138
7 AMD3516	158	0.7	9.8	225
8 AMD3530	175	0.5	2.0	350
9 AMD3517	153	0.8	10.6	191
10 AMD3544	222	0.7	3.7	317
11 AMD3543	239	0.2	1.0	1×10^3
12 AMD3529	130	0.4	2.6	325

In this field of study, it is considered that any compound exhibiting a Selectivity Index of greater than 100 has the considerable potential for further study. HIV is one of the most challenging viruses to combat, and the results given above provide an indication of activity against other retroviruses and against other viruses in general.

Example 13N-[4-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine
(AMD 7049)

5

N,N'-Bis(2-Nitrobenzenesulfonyl)-1,7-heptanediamine

To a stirred solution of 1,7-heptanediamine (5.01 g, 38.5 mmol) and Et₃N (13.5 mL, 96.9 mmol) in CH₂Cl₂ (70 mL) was added a solution of 2-nitrobenzenesulfonyl chloride (18.80 g, 84.83 mmol) in CH₂Cl₂ (40 mL). The mixture was stirred at room temperature under
10 nitrogen for 72 h and then concentrated *in vacuo*. The residue was stirred in diethyl ether (100 mL), and the precipitate was collected by filtration and washed with H₂O (300 mL) followed by diethyl ether (300 mL) to give a gray solid (18.5 g, 96%): ¹H NMR (DMF-*d*₇) δ 1.21 (m, 6H), 1.49 (m, 4H), 3.04 (m, 4H), 7.87 (m, 2H), 7.95 (m, 4H), 8.04 (m, 2H), 8.15 (m, 2H).

15

General Procedure D4-Diethoxyphosphoryl-1,7-bis(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane

To a stirred solution of N,N'-bis(2-nitrobenzenesulfonyl)-1,7-heptanediamine (9.00 g, 18.0 mmol) and Cs₂CO₃ (17.8 g, 54.6 mmol) in DMF (500 mL) under nitrogen maintained at 80 °C was added dropwise a solution of N-(diethoxyphosphoryl)-O,O'-bis(2-
20 methylsulfonyl)di-ethanolamine (Bridger et al., J. Med. Chem. 1995, 38, 366-378) (7.95 g, 20.0 mmol) in DMF (50 mL) over 8 h. Heating was continued for a further 17 h and the mixture was then allowed to cool and concentrated *in vacuo*. The residue was partitioned between CHCl₃ (140 mL) and H₂O (80 mL) and the aqueous layer was separated and extracted with CHCl₃ (3 × 40 mL). The combined organic extracts were dried (MgSO₄) and concentrated *in vacuo* and the
25 residue was purified by column chromatography on silica gel (ethylacetate) to give the desired macrocycle as a yellow crystalline solid (2.85 g, contaminated with DMF).

To remove the unwanted DMF impurity, the residue was dissolved in EtOAc (75 mL), and the solution was washed sequentially with 5% NaHCO₃ (2 × 10 mL) and brine (5 × 10 mL), dried (MgSO₄) and evaporated to give a yellow amorphous solid (2.52 g, 20%): ¹H NMR
30 (CDCl₃) δ 1.32 (t, 6H, *J* = 7.1 Hz), 1.51 (m, 6H), 1.61 (m, 4H), 3.33 (m, 12H), 4.03 (m, 4H), 7.61 (m, 2H), 7.71 (m, 4H), 8.03 (m, 2H).

General Procedure ESynthesis of 1,7-Bis(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane

To a stirred suspension of the macrocycle from above (1.88 g, 2.66 mmol) in
5 acetic acid (5 mL) was added a freshly prepared solution of saturated HBr(g) in acetic acid (20
mL) and the resulting homogeneous solution was stirred at room temperature for a further 22 h.
Addition of diethyl ether (250 mL) to the reaction mixture gave a precipitate that was allowed
to settle to the bottom of the flask and the supernatant solution was decanted. The precipitate
was washed with ether by decantation (repeated 3×) and the residue was then partitioned
10 between CH₂Cl₂ (40 mL) and 1N aqueous NaOH (25 mL). The separated aqueous layer was
extracted with CH₂Cl₂ (2 × 20 mL) and the combined organic extracts were washed with brine
(20 mL), then dried (MgSO₄) and concentrated *in vacuo* to give a yellow amorphous solid (1.23
g, 81%): ¹H NMR (CDCl₃) δ 1.46-1.67 (m, 10H), 2.90 (m, 4H), 3.34 (m, 8H), 7.61 (m, 2H),
7.70 (m, 4H), 7.97 (m, 2H).

15 4-Bromomethylbenzyl alcohol

To a solution of methyl 4-bromomethylbenzoate (5.73 g, 25 mmol) in dry CH₂Cl₂ (150
mL) cooled to -78 °C with stirring under nitrogen was added dropwise a solution of DIBAL-H
(82.5 mL, 1.0 M solution in THF). Stirring was continued for 1.5 h at -78 °C, and the reaction
mixture was then allowed to warm to 0 °C and quenched with H₂O. The organic layer was
20 separated and the aqueous was extracted with CH₂Cl₂ (2 x 100 mL). The combined organic
extracts were dried (MgSO₄) and evaporated to give the desired alcohol (5.0 g, 100%) as a
white solid: ¹H NMR (CDCl₃) δ 1.84 (br, 1H), 4.49 (s, 2H), 4.67 (s, 2H), 7.33 (d, 2H, *J* = 8.2
Hz), 7.38 (d, 2H, *J* = 8.2 Hz).

25 N-(2-Nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

A solution of 2-nitrobenzenesulfonylchloride (16.62 g, 0.075 mol) in dry CH₂Cl₂ (120
mL) was added dropwise via cannula to a stirred solution of 2-(aminomethyl)pyridine (5.41 g,
0.05 mol) and Et₃N (13.9 mL, 0.10 mol) in dry CH₂Cl₂ (150 mL) under nitrogen. The reaction
mixture was stirred for three hours at room temperature, and then quenched with water (20 mL).
30 The aqueous layer was separated and extracted with EtOAc (5 x 80 mL). The combined
organic extracts were dried (MgSO₄) and evaporated to small volume to give a white precipitate

which was collected by filtration and washed with cold CH₂Cl₂ to give the desired product (11.37 g, 78%) as a white solid: ¹H NMR (Acetone-d₆) δ 4.46 (s, 2H), 7.19 (dd, 1H, *J* = 7.4, 4.5 Hz), 7.25-7.35 (br s, 1H), 7.39 (d, 1H, *J* = 7.7 Hz), 7.68 (ddd, 1H, *J* = 7.7, 7.5, 1.8 Hz), 7.76-7.88 (m, 2H), 7.94 (dd, 1H, *J* = 7.7, 1.5 Hz), 8.04 (dd, 1H, *J* = 7.5, 1.8 Hz), 8.38 (d, 1H, *J* = 4.5 Hz).

N-[1-Methylene-4-(hydroxymethylene)phenylene]-N-(2-Nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

A mixture of N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (5.87 g, 20 mmol), 4-bromomethylbenzyl alcohol (4.02 g, 20 mmol) and K₂CO₃ (5.53 g, 40 mmol) in dry CH₃CN (150 mL) were heated at 60 °C for 4 h with stirring under nitrogen. The mixture was then allowed to cool to room temperature, the solvent evaporated and the residue was partitioned between water and CH₂Cl₂. The separated aqueous phase was extracted with CH₂Cl₂, and the combined organic extracts were dried (MgSO₄) and evaporated. The residue was suspended in ethyl acetate/hexane (1:1) and collected by filtration to give the desired product (6.87 g, 83%) as a white solid: ¹H NMR (CDCl₃) δ 1.78 (t, 1H, *J* = 5.8 Hz), 4.58 (s, 2H), 4.60 (s, 2H), 4.64 (d, 2H, *J* = 5.8 Hz), 7.13-7.26 (m, 6H), 7.54-7.59 (m, 2H), 7.66-7.68 (m, 2H), 7.98 (d, 1H, *J* = 7.4 Hz), 8.40 (d, 1H, *J* = 3.8 Hz).

N-[1-Methylene-4-(chloromethylene)phenylene]-N-(2-Nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

To a stirred solution of the alcohol from above (1.91 g, 4.62 mmol) and Et₃N (2.0 mL, 14 mmol) in CH₂Cl₂ (20 mL) cooled in an ice bath under nitrogen, was added methanesulfonyl chloride (0.73 mL, 9.4 mmol) and the reaction mixture was then heated to reflux for a further 6 h. The solution was diluted with CH₂Cl₂ (60 mL) and washed with 10% aqueous HCl (2 × 20 mL), 5% aqueous NaHCO₃ (20 mL), and H₂O (25 mL) then dried (MgSO₄) and concentrated *in vacuo* to give an orange oil (1.95 g, 98%): ¹H NMR (CDCl₃) δ 4.52 (s, 2H), 4.60 (s, 4H), 7.12-7.26 (m, 6H), 7.55 (m, 2H), 7.67 (d, 2H, *J* = 4.0 Hz), 7.94 (d, 1H, *J* = 8.0 Hz), 8.41 (d, 1H, *J* = 4.8 Hz). This was used without further purification.

30

General Procedure F

N-[4-[1,7-Bis(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetra-decanyl]-1,4-

phenylenebis(methylene)]-N-(2-Nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

A mixture of 1,7-bis(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane (1.1 g, 1.9 mmol), the chloride from above (0.98 g, 2.3 mmol) and K_2CO_3 (0.85 g, 6.2 mmol) were heated to reflux in CH_3CN (30 mL) under nitrogen for 62 h. The solvent was evaporated in vacuo and the residue was partitioned between CH_2Cl_2 (100 mL) and brine (70 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (40 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (3% MeOH/ CH_2Cl_2) and the evaporated fractions containing the desired product were subjected to a second column purification on silica gel (ethyl acetate) to give a pale yellow amorphous solid (940 mg, 49%): 1H NMR ($CDCl_3$) δ 1.44 (br s, 6H), 1.60 (br s, 4H), 2.75 (m, 4H), 3.23-3.33 (m, 8H), 3.59 (s, 2H), 4.58 (s, 2H), 4.59 (s, 2H), 7.08-7.20 (m, 6H), 7.55-7.70 (m, 10H), 7.82 (dd, 2H, $J = 7.6, 1.6$ Hz), 7.99 (d, 1H, $J = 7.8$ Hz), 8.40 (d, 1H, $J = 4.7$ Hz).

N-[4-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Pentahydrobromide Dihydrate

The intermediate from above (870 mg, 0.90 mmol), K_2CO_3 (1.15 g, 8.32 mmol), and thiophenol (0.33 mL, 3.2 mmol) were stirred in DMF (12 mL) for 7.5 h at room temperature. The mixture was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (30 mL) and H_2O (15 mL). The organic phase was separated, washed with 5% $NaHCO_3$ (10 mL) then H_2O (10 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The yellow residue was purified by column chromatography on basic alumina (CH_2Cl_2 , 1% MeOH/ CH_2Cl_2 , and 10% MeOH/ CH_2Cl_2) to give the free base as a yellow oil (134 mg, 36%): 1H NMR ($CDCl_3$) δ 1.48 (br s, 6H), 1.60 (br s, 4H), 2.61 (m, 12H), 3.56 (s, 2H), 3.83 (s, 2H), 3.92 (s, 2H), 7.16 (m, 1H), 7.24 (m, 2H), 7.32 (m, 3H), 7.79 (m, 1H), 8.56 (d, 1H, $J = 4.7$ Hz).

The free base (134 mg, 0.33 mmol) was dissolved in EtOH (4 mL) and a freshly prepared solution of saturated HBr(g) in EtOH (9 mL) was added, giving a white precipitate. The mixture was stirred for 5 min and diethyl ether (15 mL) was added. The precipitate was allowed to settle to the bottom of the flask and the supernatant solution was decanted. The solid was then dissolved in MeOH (5 mL) and re-precipitated with a large volume of ether, washed with ether by decantation (15x) and finally, the last traces of ether were removed by evaporation at reduced pressure (room temperature). Drying the solid *in vacuo* at 40 °C for 16 h, gave the

desired product as a white solid (178 mg, 63%): ^1H NMR (DMSO- d_6) δ 1.44 (br s, 6H), 1.75 (br s, 4H), 3.04 (br s, 8H), 3.37 (m, 4H), 4.06 (br s, 2H), 4.31 (s, 2H), 4.38 (s, 2H), 7.52-7.68 (m, 6H), 8.01 (m, 1H), 8.70 (d, 1H, $J = 5.0$ Hz); FAB-MS m/z 492 (MH + H^{81}Br), 490 (MH + H^{79}Br), 410 (M + H). Anal. Calcd for $\text{C}_{25}\text{H}_{39}\text{N}_5 \cdot 5\text{HBr} \cdot 0.1\text{Et}_2\text{O} \cdot 2.3\text{H}_2\text{O}$: C, 35.35; H, 5.79; N, 8.11; Br, 46.29. Found: C, 35.55; H, 5.70; N, 8.18; Br, 46.17.

Example 14

N-[7-(4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7050)

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2,6-Bis(2-aminoethyl)pyridine was prepared as described in Bridger et al. U.S. Pat. No. 5,698,546, which is hereby incorporated in its entirety by reference herein.

2,6-Bis[N-(2-nitrobenzenesulfonyl)-2-aminoethyl]pyridine

To a stirred solution of 2,6-Bis(2-aminoethyl)pyridine (2.7 g, 16 mmol) and Et_3N (5.7 mL, 41 mmol) in CH_2Cl_2 (35 mL) was added 2-nitrobenzenesulfonyl chloride (8.01 g, 36.1 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred at room temperature under nitrogen for 42 h. The mixture was washed with brine (25 mL) and the organic phase was dried (MgSO_4) and concentrated *in vacuo*. The brown residue was purified by column chromatography on silica gel (50% then 60% THF/hexane) to give a pale yellow solid (5.2 g, 59%): ^1H NMR (CDCl_3) δ 3.01 (m, 4H), 3.52 (m, 4H), 6.38 (m, 2H), 6.94 (d, 2H, $J = 7.7$, Hz), 7.47 (t, 1H, $J = 7.7$ Hz), 7.72 (m, 4H), 7.82 (m, 2H), 8.13 (m, 2H).

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7-Diethoxyphosphoryl-4,10-Bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

Using General Procedure D: Reaction of 2,6-bis[N-(2-nitrobenzenesulfonyl)-2-aminoethyl]pyridine (5.2 g, 9.7 mmol) and N-(diethoxyphosphoryl)-O,O'-bis(2-methylsulfonyl)di-ethanolamine (4.25 g, 10.7 mmol) followed by silica gel column purification (60% then 90% THF/hexane) of the reaction products gave the title compound as a yellow amorphous solid (1.48 g, 21%): ^1H NMR (CDCl_3) δ 1.23 (t, 6H, $J = 7.1$ Hz), 2.60 (m, 4H), 2.98-3.08 (m, 8H), 3.84-3.94 (m, 8H), 7.11 (d, 2H, $J = 7.6$ Hz), 7.56-7.74 (m, 7H), 8.07 (m, 2H).

30

4,10-Bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-

1(17),13,15-triene

Using General Procedure E: Reaction of 7-diethoxyphosphoryl-4,10-bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (1.04 g, 1.4 mmol) gave the title compound as a yellow amorphous solid (744 mg, 88%): ¹H NMR (CDCl₃) δ 2.81 (m, 4H), 3.08 (m, 4H), 3.33 (m, 4H), 3.88 (m, 4H), 7.07 (d, 2H, J = 7.7 Hz), 7.54-7.71 (m, 7H), 8.02 (m, 2H).

N-[7-[4,10-Bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure F: Reaction of 4,10-bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (740 mg, 1.2 mmol) and N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (610 mg, 1.4 mmol) followed by silica gel column purification (50% then 80% THF/hexane) of the reaction products gave the title compound as a yellow amorphous solid (648 mg, 54%): ¹H NMR (CDCl₃) δ 2.26 (m, 4H), 3.03 (m, 8H), 3.37 (s, 2H), 3.94 (m, 4H), 4.56 (s, 2H), 4.57 (s, 2H), 6.95-7.17 (m, 8H), 7.52-7.72 (m, 11H), 7.85 (m, 2H), 7.98 (d, 1H, J = 7.7 Hz), 8.39 (d, 1H, J = 4.8 Hz).

General Procedure G

N-[7-(4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Hexahydrobromide Trihydrate

To a solution of N-[7-[4,10-bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (640 mg, 0.64 mmol) in DMF (9 mL) containing K₂CO₃ (806 mg, 5.83 mmol) was added thiophenol (0.24 mL, 2.3 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo and the residue partitioned between ethyl acetate (30 mL) and water (10 mL). The organic phase was separated and extracted with 5% NaHCO₃ (3 x 5 mL) then brine (5 mL). The combined aqueous phases were extracted with CH₂Cl₂ (3 x 10 mL). The combined organic extracts were dried (MgSO₄) and evaporated and the residue was purified by column chromatography on alumina (CH₂Cl₂ followed by 10% MeOH/CH₂Cl₂) to give the free base of the title compound as a

yellow oil (83 mg, 29%): ^1H NMR (CDCl_3) δ 2.57 (m, 8H), 3.01 (s, 8H), 3.36 (s, 2H), 3.78 (s, 2H), 3.92 (s, 2H), 6.64 (d, 2H, $J = 8.0$ Hz), 7.07 (m, 4H), 7.18 (m, 1H), 7.33 (d, 1H, $J = 7.7$ Hz), 7.67 (m, 2H), 8.58 (d, 1H, $J = 4.8$ Hz).

The free base (74 mg, 0.17 mmol) was dissolved in MeOH (3 mL) and a freshly prepared solution of saturated HBr(g) in MeOH (7 mL) was added giving a white precipitate. The mixture was stirred for 5 min and diethyl ether was added (10 mL), the solid was allowed to settle to the bottom of the flask and the supernatant solution decanted. The solid was washed by decantation with MeOH (5 x 5 mL) then ether (10 x 5 mL) and the last traces of ether were removed by evaporation *in vacuo* followed by drying *in vacuo* at 40 °C for 17.5 h to give the title compound as a white solid (153 mg, 93%): ^1H NMR ($\text{DMSO}-d_6$) δ 2.81 (br s, 4H), 3.28 (m, 8H), 3.61 (br s, 4H), 3.85 (s, 2H), 4.27 (s, 2H), 4.36 (s, 2H), 7.29 (d, 2H, $J = 7.7$ Hz), 7.36 (d, 2H, $J = 7.7$ Hz), 7.53 (m, 3H), 7.63 (d, 1H, $J = 7.7$ Hz), 7.80 (t, 1H, $J = 7.7$ Hz), 7.99 (m, 1H), 8.69 (d, 1H, $J = 5.3$ Hz); FAB-MS m/z 527 (MH + H^{81}Br), 525 (MH + H^{79}Br), 445 (M + H). Anal. Calcd for $\text{C}_{27}\text{H}_{36}\text{N}_6 \cdot 6\text{HBr} \cdot 3\text{H}_2\text{O}$: C, 32.95; H, 4.92; N, 8.54; Br, 48.72. Found: C, 32.75; H, 4.89; N, 8.39; Br, 48.61.

Example 15

N-[7-(4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD7051)

1,3-Phenylenebis(ethylene)diamine

To a solution of 1,3-phenylenediacetonitrile (9.37 g, 60 mmol) in CH_3OH (saturated with NH_3 , 150 mL) was added Raney-Ni (*ca.* 20 g, previously washed with CH_3OH several times) and the mixture was hydrogenated at 45 psi on a Parr apparatus for 48 h. The reaction mixture was filtered through celite and the filtrate evaporated to give the crude product (9.45 g, 96%) as a light green oil: ^1H NMR (CDCl_3) δ 0.80-1.50 (br s, 4H), 2.70-2.76 (m, 4H), 2.94-2.99 (m, 4H), 7.01-7.07 (m, 3H), 7.18-7.26 (m, 1H). This was used in the next step without further purification.

N,N'-Bis(2-Nitrobenzenesulfonyl)-1,3-Phenylenebis(ethylene)diamine

A solution of 2-nitrobenzenesulfonylchloride (19.94 g, 0.090 mol) in dry CH_2Cl_2 (70 mL) was added dropwise via cannula to a stirred solution of 1,3-phenylenebis(ethylene)diamine

(4.92 g, 0.030 mol) and Et₃N (16.7 mL, 0.12 mol) in dry CH₂Cl₂ (80 mL) under nitrogen. The reaction mixture was stirred overnight at room temperature, and then quenched with water (20 mL). The precipitate was collected by filtration and washed with H₂O, CH₃OH, and Et₂O to give the desired product (9.22 g, 58%) as a white solid: ¹H NMR (DMSO-d₆) δ 2.66 (t, 4H, *J* = 7.7 Hz), 3.08-3.18 (br s, 4H), 6.94 (d, 2H, *J* = 6.4 Hz), 6.98 (s, 1H), 7.12 (dd, 1H, *J* = 6.4, 6.4 Hz), 7.78-7.84 (br m, 4H), 7.90-7.64 (br m, 4H), 8.16 (br s, 2H).

7-Diethoxyphosphoryl-4,10-bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

10 Using General Procedure D: Reaction of N,N'-bis(2-nitrobenzenesulfonyl)-1,3-phenylenebis(ethylene)diamine (8.74 g, 16.4 mmol) with N-(diethoxyphosphoryl)-O,O'-bis(2-methylsulfonyl)di-ethanolamine (6.50 g, 16.4 mmol) followed by silica gel column purification of the reaction products (1:15:35 CH₃OH-Et₂O-CH₂Cl₂) gave the title compound (4.03 g, 33%) as a yellow foam: ¹H NMR (CDCl₃) δ 1.21 (t, 6H, *J* = 6.4 Hz), 2.39-2.46 (br m, 4H), 2.83-2.97 (br m, 8H), 3.68-3.72 (m, 4H), 3.80-3.92 (m, 4H), 7.16 (d, 2H, *J* = 6.5 Hz), 7.18 (s, 1H), 7.24 (dd, 1H, *J* = 6.5, 6.5 Hz), 7.60-7.74 (m, 6H), 8.04-8.08 (m, 2H).

4,10-Bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

20 Using General Procedure E: Reaction of 7-diethoxyphosphoryl-4,10-bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (1.27 g, 1.72 mmol) followed by silica gel column purification of the reaction products (1:15:25 CH₃OH-EtOAc-CH₂Cl₂ then 20% CH₃OH in CH₂Cl₂) gave the title compound (574 mg, 57%) as a light yellow foam: ¹H NMR (CDCl₃) δ 1.42-1.50 (br, 1H), 2.01 (t, 4H, *J* = 5.4 Hz), 2.90-3.10 (br m, 4H), 3.08 (t, 4H, *J* = 5.4 Hz), 3.56-3.60 (br m, 4H), 7.16 (d, 2H, *J* = 6.8 Hz), 7.31 (dd, 1H, *J* = 6.8, 6.8 Hz), 7.36 (s, 1H), 7.61-7.63 (m, 2H), 7.70-7.73 (m, 4H), 8.01-8.04 (m, 2H).

N-[7-[4,10-Bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-Nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

30 Using General Procedure F: Reaction of 4,10-bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (420 mg, 0.7 mmol) with N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (302 mg,

0.7 mmol) followed by silica gel column purification of the reaction products (1:3 Et₂O-CH₂Cl₂) gave the title compound (491 mg, 70%) as a pale yellow solid: ¹H NMR (CDCl₃) δ 1.97-2.02 (br m, 4H), 2.73-2.78 (br m, 4H), 2.90-2.94 (br m, 4H), 3.32 (s, 2H), 3.64-3.67 (br m, 4H), 4.55 (s, 2H), 4.58 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.04 (d, 2H, *J* = 8.0 Hz), 7.09-7.16 (br m, 4H),
5 7.23 (s, 1H), 7.29 (dd, 1H, *J* = 7.9, 7.9 Hz), 7.51-7.72 (m, 10H), 7.80-7.83 (m, 2H), 7.98 (d, 1H, *J* = 7.8 Hz), 8.39 (m, 1H).

N-[7-(4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Pentahydrobromide Dihydrate

Using General Procedure G: Reaction of N-[7-[4,10-bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (380 mg, 0.38 mmol) followed by basic alumina column purification of the reaction products (1:20 CH₃OH-CH₂Cl₂) gave the free base of the title compound.

Conversion of the free base to the hydrobromide salt using a saturated solution of
15 HBr(g) in CH₃OH followed by drying *in vacuo* overnight, gave the title compound (110 mg, 34% overall) as a white solid: ¹H NMR (DMSO-d₆) δ 2.80-2.88 (br s, 4H), 3.02-3.06 (br s, 4H), 3.10-3.16 (br s, 4H), 3.38-3.44 (br s, 4H), 3.80-3.86 (br s, 2H), 4.25-4.30 (br s, 2H), 4.33-4.37 (br s, 2H), 7.27-7.32 (br m, 4H), 7.42-7.63 (br m, 6H), 7.96 (dd, 1H, *J* = 7.7, 7.7 Hz), 8.10-8.30 (br s, 3H), 8.69 (d, 1H, *J* = 4.9 Hz), 9.45-9.62 (br s, 2H); FAB-MS *m/z* 526 (MH + H⁸¹Br), 524 (MH + H⁷⁹Br), 444 (M + H, 100); Anal. Calcd for C₂₈H₄₂N₅Br₃•2H₂O: C, 38.03; H, 5.24; N, 7.92; Br, 45.18. Found: C, 38.37; H, 5.28; N, 7.76; Br, 45.36.

Example 16

N-[1-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7059)

25

General Procedure H

4-Diethoxyphosphoryl-7-(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane

To a stirred solution of 4-diethoxyphosphoryl-1,7-bis(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane (1.32 g, 1.87 mmol) and K₂CO₃ (654 mg, 4.73 mmol) in DMF (11 mL)
30 under nitrogen was added dropwise a solution of thiophenol (0.15 mL, 1.46 mmol) in DMF (8 mL) over 1 h. The mixture was stirred for an additional 3 h and then concentrated *in vacuo*.

The residue was partitioned between CHCl_3 (50 mL) and H_2O (25 mL). The aqueous phase was separated and extracted with CHCl_3 (3×20 mL) and the combined organic extracts were dried (MgSO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on basic alumina (CHCl_3 then 3% $\text{MeOH}/\text{CHCl}_3$) to give the title compound as a yellow oil (178 mg, 23%): ^1H NMR (CDCl_3) δ 1.31 (t, 6H, $J = 7.0$ Hz), 1.40-1.67 (m, 10H), 2.65 (m, 2H), 2.78 (m, 2H), 3.12 (m, 2H), 3.26-3.37 (m, 4H), 3.48 (m, 2H), 3.97-4.09 (m, 4H), 7.61 (m, 1H), 7.68 (m, 2H), 8.06 (m, 1H).

N-[1-[4-Diethoxyphosphoryl-7-(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecanyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure F: Reaction of 4-diethoxyphosphoryl-7-(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetradecane (236 mg, 0.453 mmol) and N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (238 mg, 0.551 mmol) followed by silica gel column purification of the reaction products (50% then 80% THF/hexane) gave the title compound as a yellow amorphous solid (305 mg, 73%): ^1H NMR (CDCl_3) δ 1.27 (t, 6H, $J = 7.1$ Hz), 1.43 (br s, 8H), 1.63 (br s, 2H), 2.32 (br s, 2H), 2.55 (m, 2H), 3.13-3.41 (m, 8H), 3.49 (s, 2H), 3.85-4.02 (m, 4H), 4.57 (s, 2H), 4.58 (s, 2H), 7.07-7.22 (m, 6H), 7.51-7.71 (m, 7H), 7.98 (d, 1H, $J = 7.4$ Hz), 8.04 (m, 1H), 8.41 (d, 1H, $J = 4.0$ Hz).

N-[1-[7-(2-Nitrobenzenesulfonyl)-1,4,7-triazacyclotetra-decanyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure E: Reaction of N-[1-[4-diethoxyphosphoryl-7-(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetra-decanyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (300 mg, 0.328 mmol) gave the title compound as a yellow amorphous solid (214 mg, 84%): ^1H NMR (CDCl_3) δ 1.34-1.44 (m, 8H), 1.69 (br s, 2H), 2.34 (m, 2H), 2.52 (m, 2H), 2.62 (m, 2H), 2.82 (m, 2H), 3.42 (m, 6H), 4.58 (s, 2H), 4.59 (s, 2H), 7.08-7.24 (m, 6H), 7.52-7.71 (m, 7H), 8.01 (m, 2H), 8.42 (d, 1H, $J = 4.1$ Hz).

N-[1-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Pentahydrobromide Dihydrate

A mixture of N-[1-[7-(2-nitrobenzenesulfonyl)-1,4,7-triazacyclotetra-decanyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (209 mg, 0.268 mmol), K_2CO_3 (298 mg, 2.16 mmol), and thiophenol (0.17 mL, 1.7 mmol) in acetonitrile (3 mL) were heated to 50 °C for 16.5 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL)

and washed with brine (10 mL). The separated aqueous phase was extracted with CH₂Cl₂ (3 x 5 mL) and the combined organic extracts were dried (MgSO₄) and evaporated. The residue was purified by column chromatography on basic alumina (CHCl₃ then 10% MeOH/CHCl₃) to give the free base of title compound as a yellow oil (92 mg, 84%): ¹H NMR (CDCl₃) δ 1.21-1.62 (m, 10H), 2.40-2.49 (m, 4H), 2.60 (m, 6H), 2.79 (m, 2H), 3.49 (s, 2H), 3.80 (s, 2H), 3.91 (s, 2H), 7.14 (m, 1H), 7.28 (m, 5H), 7.62 (m, 1H), 8.54 (d, 1H, J = 4.4 Hz).

Conversion of the free base (86 mg, 0.21 mmol) to the hydrobromide salt using a saturated solution of HBr(g) in MeOH (See General Procedure G) followed by drying *in vacuo* at 40 °C for 15.5 h gave the title compound as a white solid (128 mg, 70%): ¹H NMR (D₂O) δ 1.48 (br s, 6H), 1.82 (m, 4H), 3.22-3.36 (m, 10H), 3.50 (br s, 2H), 4.48 (s, 4H), 4.64 (s, 2H), 7.62 (s, 4H), 7.88 (m, 1H), 7.94 (d, 1H, J = 8.0 Hz), 8.38 (m, 1H), 8.77 (d, 1H, J = 5.2 Hz); FAB-MS *m/z* 492 (MH + H⁸¹Br), 490 (M + H⁷⁹Br), 410 (M + H). Anal. Calcd for C₂₅H₃₉N₅·5HBr·2.5H₂O·0.1Et₂O: C, 35.20; H, 5.82; N, 8.08; Br, 46.10. Found: C, 35.48; H, 5.66; N, 8.10; Br, 46.05.

Example 17

N-[4-[4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7063)

7-Diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

Using General Procedure H: Reaction of 7-diethoxyphosphoryl-4,10-bis(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (1.48 g, 2.00 mmol) with thiophenol (with an additional heating of the reaction mixture to 50 °C for 1.5 h after the addition) followed by silica gel column purification of the reaction products (8% MeOH/CHCl₃) gave the title compound as a light yellow oil (423 mg, 52%): ¹H NMR (CDCl₃) δ 1.23 (t, 6H, J = 7.0 Hz), 2.50 (br s, 2H), 2.79 (br s, 2H), 3.02-3.15 (m, 10H), 3.82-3.98 (m, 6H), 7.06 (d, 2H, J = 7.6 Hz), 7.54-7.63 (m, 2H), 7.70 (m, 2H), 8.01 (br s, 1H).

N-[4-[7-Diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure F: Reaction of 7-diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (410 mg, 0.738 mmol) and N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (397 mg, 0.919 mmol) followed by silica gel column purification of the reaction products (50%, 80%, and 90% THF/hexane) gave the title compound as a white amorphous solid (441 mg, 63%): ¹H NMR (CDCl₃) δ 1.15 (t, 6H, *J* = 7.0 Hz), 2.42 (m, 4H), 2.77 (m, 2H), 2.92-3.02 (m, 6H), 3.10 (m, 2H), 3.59 (s, 2H), 3.66-3.91 (m, 6H), 4.58 (s, 2H), 4.59 (s, 2H), 6.94 (d, 1H, *J* = 7.6 Hz), 7.07-7.14 (m, 6H), 7.22 (d, 1H, *J* = 7.8 Hz), 7.51-7.72 (m, 8H), 8.00 (d, 1H, *J* = 7.8 Hz), 8.04 (m, 1H), 8.42 (d, 1H, *J* = 4.0 Hz).

10 N-[4-[7-Diethoxyphosphoryl-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-2-(aminomethyl)pyridine

A mixture of N-[4-[7-diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (434 mg, 0.456 mmol), K₂CO₃ (508 mg, 3.68 mmol), and thiophenol (0.28 mL, 2.7 mmol) were heated to 50 °C in CH₃CN (3.5 mL) under nitrogen for 15 h. Upon cooling, the reaction mixture was partitioned between CHCl₃ (15 mL) and brine (15 mL) and the aqueous layer was separated and extracted with CHCl₃ (3 × 5 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo and the residue was purified by column chromatography on basic alumina (CHCl₃ then 10% MeOH/CHCl₃) to give a yellow oil (218 mg, 82%): ¹H NMR (CDCl₃) δ 1.16 (t, 6H, *J* = 7.1 Hz), 2.35 (m, 2H), 2.55 (m, 2H), 2.75 (m, 2H), 2.82 (m, 2H), 2.96-3.08 (m, 6H), 3.16 (m, 2H), 3.68 (s, 2H), 3.69-3.88 (m, 4H), 3.82 (s, 2H), 3.93 (s, 2H), 6.95 (d, 1H, *J* = 7.6 Hz), 7.00 (d, 1H, *J* = 7.5 Hz), 7.15-7.34 (m, 6H), 7.50 (m, 1H), 7.65 (m, 1H), 8.56 (d, 1H, *J* = 4.7 Hz).

25 N-[4-[4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Hexahydrobromide Hydrate

To a stirred solution of N-[4-[7-diethoxyphosphoryl-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-2-(aminomethyl)pyridine (211 mg, 0.36 mmol) in acetic acid (0.6 mL) was added a freshly prepared solution of saturated HBr(g) in acetic acid (6 mL) and the reaction mixture was allowed to stir at room temperature for 4 h. Addition of diethyl ether (10 mL) gave a white precipitate that was allowed to settle to the bottom of the flask and the supernatant solution was

decanted. The solid was washed by decantation with MeOH (4 x 5 mL) and ether (6 x 5 mL) and the remaining traces of ether were removed by evaporation under reduced pressure. The product was dried in vacuo at 40 °C for 17 h, to give the title compound as a pale yellow solid (223 mg, 63%): ¹H NMR (D₂O) δ 3.14-3.36 (m, 10H), 3.55 (m, 4H), 3.75 (m, 2H), 4.45 (s, 2H), 4.50 (s, 2H), 4.64 (s, 2H), 7.22 (m, 2H), 7.53 (s, 4H), 7.70 (m, 1H), 7.95 (m, 1H), 8.00 (d, 1H, J = 7.9 Hz), 8.46 (m, 1H), 8.79 (d, 1H, J = 3.9 Hz); FAB-MS m/z 527 (MH + H⁸¹Br), 525 (MH + H⁷⁹Br), 445 (M + H). Anal. Calcd for C₂₇H₃₆N₆·6HBr·1.5H₂O·0.2Et₂O: C, 34.35; H, 4.87; N, 8.65; Br, 49.33. Found: C, 34.57; H, 5.04; N, 8.68; Br, 49.09.

Example 18

N-[4-[4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD7058)

7-Diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

Using General Procedure H: Reaction of 7-diethoxyphosphoryl-4,10-bis(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene

(1.11 g, 1.5 mmol) followed by silica gel column purification of the reaction products (2:5:20 CH₃OH-Et₂O-CH₂Cl₂ then 1:5 CH₃OH-CH₂Cl₂) gave the title compound as a pale yellow oil (300 mg, 54%): ¹H NMR (CDCl₃) δ 1.21 (t, 6H, J = 7.1 Hz), 1.78-1.92 (br s, 1H), 2.31-2.38 (br m, 2H), 2.56-2.60 (br m, 2H), 2.81-2.98 (br m, 10H), 3.60-3.64 (br m, 2H), 3.75-3.91 (m, 4H), 7.05-7.12 (m, 2H), 7.24-7.29 (m, 2H), 7.60-7.63 (m, 1H), 7.68-7.71 (m, 2H), 8.02-8.06 (m, 1H).

N-[4-[7-Diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure F: Reaction of 7-diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-triene (290 mg, 0.52 mmol) with N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (271 mg, 0.63 mmol) followed by silica gel column purification of the reaction products (1:12:12 CH₃OH-Et₂O-CH₂Cl₂) gave the title compound (298 mg, 60%) as a

pale yellow solid: ^1H NMR (CDCl_3) δ 1.17 (t, 6H, $J = 7.0$ Hz), 2.29-2.45 (br m, 4H), 2.55-2.65 (br m, 2H), 2.71-2.75 (br s, 4H), 2.85-2.91 (br m, 2H), 2.96-2.98 (br m, 2H), 3.57 (s, 2H), 3.67-3.84 (br m, 6H), 4.57-4.61 (br s, 4H), 7.07-7.28 (br m, 10H), 7.55-7.71 (br m, 7H), 7.99-8.02 (m, 2H), 8.42-8.46 (m, 1H).

5 N-[4-[10-(2-Nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine

Using General Procedure E: Reaction of N-[4-[7-diethoxyphosphoryl-10-(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (290 mg, 0.31 mmol) gave the title compound (240 mg, 95%) as a white solid: ^1H NMR (CDCl_3) δ 1.65-1.79 (br s, 1H, coincide with H_2O peak), 2.15-2.19 (br m, 4H), 2.44-2.48 (br m, 2H), 2.61-2.65 (br m, 2H), 2.67-2.71 (br m, 2H), 3.00-3.04 (br m, 2H), 3.10-3.14 (br m, 2H), 3.56-3.60 (br s, 4H), 4.55 (s, 2H), 4.61 (s, 2H), 6.96 (d, 1H, $J = 7.8$ Hz), 7.02-7.10 (br m, 6H), 7.22-7.28 (br m, 3H), 7.52-7.72 (br m, 7H), 7.96-7.99 (m, 2H), 8.42-8.46 (m, 1H). This was used without further purification.

15 N-[4-[4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine Pentahydrobromide Dihydrate

Using General Procedure G: Reaction of N-[4-[10-(2-nitrobenzenesulfonyl)-4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-N-(2-nitrobenzenesulfonyl)-2-(aminomethyl)pyridine (236 mg, 0.29 mmol) followed by alumina column purification of the reaction products (1:99 $\text{CH}_3\text{OH}-\text{CH}_2\text{Cl}_2$ then 1:10 $\text{CH}_3\text{OH}-\text{CH}_2\text{Cl}_2$) gave the free base of the title compound (111 mg, 86%) as a pale yellow oil: ^1H NMR (CDCl_3) δ 2.24-2.28 (br s, 3H), 2.43-2.50 (br m, 4H), 2.58-2.62 (br m, 2H), 2.73-2.79 (br m, 8H), 2.95-2.98 (br m, 2H), 3.50 (s, 2H), 3.77 (s, 2H), 3.90 (s, 2H), 6.83-6.87 (br m, 3H), 7.05-7.33 (br m, 7H), 7.63-7.67 (m, 1H), 8.54-8.56 (m, 1H).

Conversion of the free base (104 mg, 0.23 mmol) to the hydrobromide salt using a saturated solution of HBr(g) in CH_3OH followed by drying of the product *in vacuo*, gave the title compound (101 mg, 52%) as a white solid: ^1H NMR (D_2O) δ 2.90-2.94 (br m, 2H), 2.97-3.01 (br m, 2H), 3.12-3.16 (br m, 2H), 3.17-3.21 (br m, 2H), 3.24-3.28 (br m, 4H), 3.47-3.51 (br m, 2H), 3.57-3.61 (br m, 2H), 4.38-4.42 (m, 6H), 7.34-7.40 (m, 2H), 7.46-7.60 (m, 8H), 7.90-

7.94 (m, 1H), 8.58-8.62 (m, 1H); FAB-MS m/z 526 (MH + H⁸¹Br), 524 (MH + H⁷⁹Br), 444 (M + H, 100); Anal. Calcd. for C₂₈H₄₂N₅Br₅•2.5H₂O: C, 37.65; H, 5.30; N, 7.84; Br, 44.73. Found: C, 37.53; H, 5.26; N, 7.79; Br, 44.75.

5

Table 2

Compound	Inhibition of mAb 12G5 binding
	IC ₅₀ ^a (ng/ml)
AMD3100	27
AMD3465	3
AMD7049	52
AMD7050	1
AMD7051	7
AMD7058	>1000
AMD7059	>1000
AMD7063	9
SDF-1 α ^b	270

^a Inhibition of mAb 12G5 binding to CXCR-4 in SUP-T1 cells.

^b Natural ligand for CXCR-4 (Bleul *et al. Nature*, 382: 829-832 (1996); Oberlin *et al., Nature*, 382:833-835 (1996)).

Table 3

Compound	% Inhibition of Ca²⁺ flux (conc) or IC₅₀^a (ng/ml)
AMD3100	5
AMD3465	1
AMD7049	100% (1 µg/ml)
AMD7050	100% (1 µg/ml)
AMD7051	100% (1 µg/ml)
AMD7058	44% (1 µg/ml)
AMD7059	36% (1 µg/ml)
AMD7063	100% (1 µg/ml)

^a Inhibition of Signal transduction (increasing intracellular Ca²⁺ flux) induced by SDF-1α binding to CXCR-4 on SUP-T1 cells.

Each of the following compounds, including AMD 3484 (see Fig.28), were synthesized according to procedures in Bridger *et al.*, *J. Med. Chem.* 1995, 38, 366-378; *J. Med. Chem.* 1996, 39, 109-119 and U.S. Pat. No. 5,583,131, U.S. Pat. No. 5,698,546 and U.S. Pat. No. 5,817,807, which are each incorporated in their entirety by reference herein.

5

Example 191-[2,6-Dimethoxypyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Tetrahydrobromide (AMD 7032)

¹H NMR (D₂O) δ 1.78 (m, 2H), 1.88-1.92 (m, 2H), 2.59-3.03 (m, 16H), 3.60 (s, 2H), 3.91 (s, 6H), 6.44 (s, 2H); ¹³C NMR (D₂O) δ 26.75, 27.91, 48.34, 49.21, 49.89, 50.96, 52.01, 52.86, 54.88, 57.15, 57.53, 59.42, 142.65, 157.42, 166.42; FAB MS *m/z* 352 (M+H); Anal. (C₁₈H₃₃N₅O₂ 4.1 HBr 0.25 H₂O); Calc. C, 31.44; H, 5.51; N, 10.18; Br, 47.64. Found C, 31.17; H, 5.61; N, 9.92; Br, 47.54.

10

Example 20

15 1-[2-Chloropyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Tetrahydrochloride Monohydrate (AMD 7048)

¹H NMR (D₂O) δ 1.92 (m, 2H), 2.12 (m, 2H), 2.77-2.80 (m, 4H), 2.96-3.39 (m, 12H), 3.85 (s, 2H), 7.33 (d, 1H, *J* = 5.4 Hz), 7.44 (s, 1H), 8.40 (d, 1H, *J* = 5.4 Hz); ¹³C NMR (D₂O) δ 24.75, 27.59, 47.40, 47.55, 49.11, 49.23, 52.12, 52.40, 53.81, 54.42, 56.98, 126.97, 128.30, 151.90, 152.34, 153.78; FAB MS *m/z* 326 (M+H); Anal. (C₁₆H₂₈N₅Cl.4.2HCl.0.5HOAc.1.1H₂O); Calc. C, 38.61; H, 6.94; N, 13.24; Cl, 34.86. Found C, 38.63; H, 6.94; N, 13.52; Cl, 34.61.

20

Example 21

25 1-[2,6-Dimethylpyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Pentahydrobromide Dihydrate (AMD 7060)

¹H NMR (D₂O) δ 1.77 (m, 2H), 1.93 (m, 2H), 2.48 (s, 6H), 2.61-3.00 (m, 16H), 3.61 (s, 2H), 7.07 (s, 2H); ¹³C NMR (D₂O) δ 25.30, 26.22, 27.49, 47.75, 48.65, 49.43, 50.41, 51.58, 52.19, 54.09, 56.63, 58.46; FAB MS *m/z* 320 (M+H); Anal. (C₁₈H₃₃N₅.5HBr.0.5HOAc.1.7H₂O); Calc. C, 29.08; H, 5.57; N, 8.92; Br, 50.91. Found C, 29.04; H, 5.60; N, 8.73; Br, 50.87.

30

Example 22

35 1-[2-Methylpyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Pentahydrobromide Dihydrate (AMD 7061)

¹H NMR (D₂O) δ 2.01-2.08 (m, 2H), 2.22 (m, 2H), 2.70-2.72 (m, 2H), 2.77 (s, 3H), 2.91-2.92 (m, 2H), 3.33-3.52 (m, 12H), 4.00 (s, 2H), 7.86-7.89 (m, 2H), 8.56 (d, 1H, *J* = 5.7 Hz); FAB MS *m/z* 306 (M+H); Anal. (C₁₇H₃₁N₅.4.9HBr.0.3HOAc.2.1 H₂O); Calc. C, 27.9; H, 5.49; N, 9.24; Br, 51.67. Found C, 28.08; H, 5.50; N, 9.56; Br, 51.56.

40

Example 231-[2,6-Dichloropyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Trihydrochloride Bishydrate (AMD 3451)

5 ^1H NMR (D_2O) δ 1.83-1.88 (m, 2H), 2.04-2.08 (m, 2H), 2.58-2.62 (m, 2H), 2.79-2.81 (m, 2H), 3.12-3.44 (m, 12H), 3.69 (s, 2H), 7.30 (s, 2H); ^{13}C NMR (D_2O) δ 36.26, 37.69, 55.26, 56.18, 58.33, 58.56, 58.92, 59.23, 63.57, 65.44, 70.72, 140.37, 166.14, 167.37; FAB MS m/z 360 (M+H). Anal. ($\text{C}_{16}\text{H}_{34}\text{N}_5\text{Cl}_5\text{O}_2$): Calc. C, 38.00; H, 6.78; N, 13.85; Cl, 35.05. Found: C, 38.33; H, 6.42; N, 13.88; Cl, 35.43.

10

Example 241-[2-Chloropyrid-5-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane Tetrahydrochloride Hemihydrate (AMD 3454)

15 ^1H NMR (D_2O) δ 1.96-2.09 (br m, 4H), 3.02-3.17 (m, 4H), 3.19-3.28 (br m, 8H), 3.40 (s, 4H), 4.10 (s, 2H), 7.40 (d, 1H, $J = 8.2$ Hz), 7.80 (d, 1H, $J = 8.2$ Hz), 8.27 (s, 1H); ^{13}C NMR (D_2O) δ 19.36, 19.47, 38.17, 38.64, 39.06, 41.74, 41.88, 42.18, 45.66, 48.29, 54.62, 125.59, 126.69, 142.79, 150.77, 151.75; FAB-MS m/z 326 (M + H). Anal. Calcd for $\text{C}_{16}\text{H}_{28}\text{N}_5\text{Cl}_4\text{HCl} \cdot 0.5\text{H}_2\text{O}$: C, 39.98; H, 6.92; N, 14.57; Cl, 36.87. Found: C, 40.36; H, 7.06; N, 14.56; Cl, 36.92.

20

General Procedures A, B and C were used to prepare the following compounds:

Example 25N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-purine Pentahydrobromide DiHydrate (AMD3472)

25

^1H NMR (D_2O) δ 1.88-2.05 (br m, 4H), 3.06-3.22 (br m, 8H), 3.27-3.44 (br m, 8H), 4.22 (s, 2H), 5.59 (s, 2H), 7.29 (s, 4H), 8.80 (s, 1H), 9.11 (s, 1H), 9.28 (s, 1H); ^{13}C NMR (D_2O) δ 18.39, 19.25, 37.24, 37.55, 37.71, 41.13, 41.37, 41.71, 44.41, 47.73, 54.87, 129.45, 131.81, 132.53, 136.67, 140.96, 147.88, 152.46, 154.37; FAB-MS m/z 423 (M + H). Anal. Calcd for $\text{C}_{23}\text{H}_{34}\text{N}_8 \cdot 5\text{HBr} \cdot 2\text{H}_2\text{O} \cdot 0.5\text{CH}_3\text{CO}_2\text{H}$: C, 32.27; H, 5.07; N, 12.54; Br, 44.73. Found: C, 32.66; H, 4.81; N, 12.41; Br, 44.58.

30

Example 261-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-phenylpiperazine Pentahydrobromide Hydrate (AMD3526)

35

¹H NMR (D₂O) δ 1.88-2.06 (br m, 4H), 3.11-3.53 (br m, 24H), 4.30 (s, 2H), 4.32 (s, 2H), 6.89-6.97 (m, 3H), 7.19-7.24 (m, 2H), 7.49 (s, 4H); ¹³C NMR (D₂O) δ 18.74, 19.37, 37.34, 41.47, 41.76, 42.03, 44.31, 47.45, 48.26, 51.16, 58.48, 59.29, 118.18, 122.34, 129.99, 130.37, 131.53, 131.85, 132.62, 148.47; FAB-MS m/z 547 (M + H⁸¹Br), 545 (M + H⁷⁹Br), 465 (M + H). Anal. Calcd for C₂₈H₄₄N₆.5HBr.H₂O: C, 37.90; H, 5.79; N, 9.47; Br, 45.03. Found: C, 37.72; H, 5.98; N, 9.38; Br, 46.78.

Table 4

Compound	IC ₅₀ ^a (μg/mL)
AMD3451	8.9
AMD3472	45.4
AMD3454	32.3
AMD3526	82
AMD3100	>100

^a 50% Inhibitory Concentration (IC₅₀)(μg/mL) exhibited by AMD compounds against infection of U87.CD4.CCR5 by HIV-1 BaL

Example 27

Inhibition of Collagen-Induced Arthritis

A compound as used in the present invention demonstrated inhibition of collagen-induced arthritis (CIA) in a mutant mouse model.

METHODOLOGY

Experimental Animal Treatment

The control group consisted of ten mice that were injected with collagen as discussed below. The treatment group consisted of eight mice which were also injected with collagen and were further treated by administering a compound of (1,1'-[1,4-phenylenebis(methylene)]]bis-1,4,8,11-tetraazacyclotetradecane) (AMD 3100; see figure 27) intravenously using osmotic pumps (200μl, Alza, 0.5 μl/hr) at a concentration of 5 mg/ml over a 14-day period following collagen injection.

Mutant Mice

The generation and the basic characteristics of the mutant mouse strain (129/Sv/Ev) with a disruption in the gene coding for the α -chain of the IFN- γ receptor (IFN- γ RKO) have been described (Huang S. *et al.*, *Science* 259:1742, (1993)). These IFN- γ RKO mice were back-crossed with DBA/1 wild-type mice for 10 generations to obtain the DBA/1 IFN- γ RKO mice used in the present study. IFN- γ RKO and wild-type mice were bred in the Experimental Animal Centre of the University of Leuven. The experiments were performed in 8- to 12-week old mice, but in each experiment, the mutant and wild-type mice were age-matched with a 5 day limit. The male to female ratio was kept between 0.8 and 1.3 in each experimental group.

Induction of Collagen-Induced Arthritis and Clinical Assessment of Arthritis

Collagen-induced arthritis was carried out in the following manner (see: Vermeire *et al.*, *Int. J. Immunol.* 158:5507-5513, (1997)). Native chicken collagen type II (EPC, Owensville, MO) was dissolved in 0.05 M acetic acid at 2 mg/ml by stirring overnight at 6°C, and emulsified in an equal volume of incomplete (IFA) or complete Freund's adjuvant (CFA) containing 1.5 mg/ml heat killed *Mycobacterium butyricum* (Difco, Detroit, MI). Mice were sensitized with a single 100 μ l intradermal injection of the emulsion at the base of the tail. Mice were examined daily for signs of arthritis. The disease severity was recorded following a scoring system for each limb. Score 0: normal; score 1: redness and /or swelling in one joint; score 2: redness and/or swelling in more than one joint; score 3: redness and/or swelling in the entire paw; score 4: deformity and/or ankylosis.

Histological Examination

Spleens and fore and hind limbs were fixed in buffered saline - B5fixative (10% formalin with quicksilver). Alternatively, tissues were fixed in 10% formalin or pure methanol (see: Vermeire *et al.*, *J. Immunol.*, 158, 5507-5513, 1997). Limbs were subsequently decalcified overnight with formic acid. Four-micron thick paraffin sections were stained with hematoxylin and eosin. Severity of arthritis was evaluated using three parameters: infiltration of mono- and polymorphonuclear cells, hyperplasia of the synovium and pannus formation. Each parameter was scored on a scale from 0 to 3 (absent; weak, moderate and severe).

In Vivo Antibody Treatments

Monoclonal antibodies were produced from hybridomas grown by intraperitoneal inoculation in pristane-primed athymic nude mice (nu/nu of NMRI background). Neutralizing monoclonal antibody against MuIFN- γ (F3, rat IgG_{2a}) was purified by affinity chromatography

on a mouse anti-rat κ chain monoclonal antibody (Billiau A. *et al.*, *J. Immunol.* 140:1506, (1988)). The neutralizing titer (end-point dilution corresponding to 50% neutralization of the antiviral effect of 30 units/ml of mouse IFN- γ on mouse I929 cells challenged with mengovirus) was 10^{53} U/ml (IgG content, 1.4 mg/ml). A neutralizing rate IgG₂₄ antibody against murine IL-12 was produced using hybridoma C17.8 (kindly provided by Dr. G. Trinchieri, Wistar Institute, Philadelphia, PA). The antibody was purified by affinity chromatography on protein G (Pharmacia, Uppsala, Sweden). Antibody against murine IL-6 was prepared from ascites fluid from thymus-less nude mice inoculated with the 20F3 (rat x mouse) hybridoma (American Type Culture Collection, Rockville, MD). This rat IgG antibody was purified by affinity chromatography on an anti-rat κ chain monoclonal antibody-Sepharose column. The neutralizing titer (endpoint dilution corresponding to 50% neutralization of the cell growth effect of 10 U of murine IL-6 per ml) was 10^{55} (IgG content: 2.9 mg/ml).. Irrelevant rat IgG₂₄ was used as an isotope control and was prepared from ascites fluid of a rat plasmocytoma (obtained through the courtesy of Dr. H. Bazin, University of Louvain, Medical School, Brussels, Belgium). The IgG was purified by anion exchange chromatography on Hiload Q Sepharose and gel filtration on Superdex 200 (Pharmacia). Batches of anti-IFN- γ , anti-IL-12, anti-IL-6 and irrelevant IgG₂₄ were tested for endotoxin content by a chromogenic *Limulus* amoebocyte lysate assay (KabiVitrum, Stockholm, Sweden) and were found to contain less than 2 ng/ml endotoxin. Injections were given in 200 μ l of pyrogen-free saline.

RESULTS

Following 14 days after treatment, 7 of the ten mice in the control group demonstrated arthritis, while only 1 of the 8 treated animals demonstrated disease. See Figure 29. The single treated animal did not develop arthritic pathology until after 20 days post-treatment. Additionally, the treated animals compared with the control animals did not demonstrate any significant body weight loss. See Figure 30. Further, the treated animals maintained body weight consist with healthy animals not injected with collagen (curve not shown).

Example 28

Treatment of Glioblastoma

Compounds of the present invention, such as AMD 3100 may be used in the treatment of glioblastomas, fibromas, astrocytomas or myelomas affecting the central nervous system.

The compounds may be used according to standard clinical practice and procedures, using dosages as provided in the foregoing examples and according to clinical end points, such as imaging, immunological and other methodologies.

For example, the etiology or association of chemokine receptor binding in the proliferation of glioblastoma tumor cells has been reported by Sehgal *et al.*, *J. of Surg. Oncolo.* 69:99-104 (1998) ("Sehgal I") and Sehgal *et al.*, *J. of Surg. Oncolo.* 69:239-248 (1998) ("Sehgal II"). The role of CXCR4 of its binding to its receptor appears to play a significant role in the formation and/or proliferation of glioblastoma cells. The inhibition of the binding by CXCR4 to its natural receptor ligand by compounds of the present invention, such as AMD 3100, offer a new drug in the treatment tumors of central nervous system that are mediated or associated with chemokines, such as CXCR4.

Example 29

Treatment of Non-Small Cell Lung Cancer

Compounds of the present invention, such as AMD 3100, may be used in the treatment of non-small cell lung cancer. The compounds may be used according to standard clinical practice and procedures, using dosages as provided in the foregoing examples and according to clinical end points, such as imaging, immunological and other methodologies.

For example, CXC chemokines have been found to regulate or are associated with the regulation of angiogenesis in non-small cell lung cancer (see: Arenberg, *et al.*, *J. of Leukocyte Biol.*; 62:554562 (1997); and Moore *et al. TCM*, vol 8(2): 51-58 (1998) Elsevier Science, Inc.). The role of CXC chemokines and the binding to their respective receptors appear to play a significant role in the formation and/or proliferation of non-small cell lung cancer promoted by an increase in angiogenic activity. The inhibition of the binding of these CXC chemokines to their natural receptor ligands by compounds of the present invention, such as AMD 3100, offer a new drug in the treatment tumors such as non-small cell lung cancer that are mediated or associated with increased levels of chemokines.

Example 30

N-[4-(1,7-Diazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine

The compounds of the present invention further include compounds of formula I, where V has 2 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other. An example of such compound, includes:

N-[4-(1,7-Diazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD-Exp 1; see Figure 34).

AMD-Exp 1, Exp-2 and Exp3 may each be prepared by modification of our previously published strategies (Bridger *et al. J. Med. Chem.* 1995, 38, 366) for preparing carbon-linked azamacrocycles and is briefly summarized as follows: The aforementioned intermediate, N-[1-methylene-4-(chloromethylene)phenylene]-N-(2-nitrobenzenesulfonyl)-2-aminomethylpyridine (or the Dep -protected intermediate) is reacted with the sodium salt of diethylmalonate to give the corresponding diester.

Reduction of the diester to the corresponding diol, followed by derivatization with methanesulfonyl chloride gave the dimesylate. Nucleophilic displacement of the dimesylate with sodium cyanide gives the requisite dinitrile which was reduced to the diamine with borane.THF and derivatized with 2-nitrobenzenesulfonyl chloride for the impending macrocyclization reaction.

Macrocyclization with an appropriately derivatized diol (such as 1,7-heptanediol di-p-tosylate) and subsequent deprotection as described in previous examples gave, for example, AMD-Exp 1. Similarly, 1,3-phenylenediethanol and 2,6-pyridine diethanol would give AMD-Exp 2 and AMD-Exp-3 respectively.

Further, AMD-Exp 1, AMD-Exp 2, and AMD-Exp 3 may each be prepared into a pharmaceutical compositions comprising a therapeutically effective amount of the compound in a pharmaceutically acceptable carrier.

Additionally, AMD-Exp 1, AMD-Exp 2, and AMD-Exp 3 may each be used according to the foregoing methods for the treatment of a number of chemokine-mediated diseases and conditions, including: infection with HIV or FIV; a disease by the regulation of endothelial cell function; a disease relating to vascularization of the gastrointestinal tract; haematopoiesis; or cerebellar development; a disease relating to basal leukocyte trafficking or the extravasation and tissue infiltration of leukocytes in response to inciting antigens; compound effectively binds to a chemokine receptor; of inflammatory disease; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; cardiogenesis developmental disease;

haematopoiesis and other chemokine mediated diseases or disorders.

Additional examples of such specific diseases or conditions that may be treated using AMD-Exp 1, further include: inflammation due to arthritis or multiple sclerosis; cancers associated with: solid tumors; lymphoma; metastatic tumors; glioblastoma tumors; and other carcinoma tumors; non-small cell lung cancer; lung cancer; breast cancer; prostate cancer; and cancer of other organs. Further, disorders or conditions that may be treated using AMD-Exp 1, includes: disorders treated by inhibiting or promoting angiogenesis or by inducing stasis of angiogenesis.

Example 31

N-[7-(4,10-Diazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine

The compounds of the present invention further include compounds of formula I, where V has 2 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other. An example of such compound, includes:

N-[7-(4,10-Diazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD-Exp 2; see Figure 36).

AMD-Exp 2 and AMD-Exp 3 may each be prepared according to Example 30 above. Further, AMD-Exp 2 may be prepared into a pharmaceutical compositions comprising a therapeutically effective amount of the compound in a pharmaceutically acceptable carrier.

Additionally, AMD-Exp 2 may be used according to the foregoing methods for the treatment of a number of chemokine-mediated diseases and conditions, including: infection with HIV or FIV; a disease by the regulation of endothelial cell function; a disease relating to vascularization of the gastrointestinal tract; haematopoiesis; or cerebellar development; a disease relating to basal leukocyte trafficking or the extravasation and tissue infiltration of leukocytes in response to inciting antigens; compound effectively binds to a chemokine receptor; of inflammatory disease; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; cardiogenesis developmental disease; haematopoiesis and other chemokine mediated diseases or disorders.

Additional examples of such specific diseases or conditions that may be treated using AMD-Exp 2, further include: inflammation due to arthritis or multiple sclerosis;

cancers associated with: solid tumors; lymphoma; metastatic tumors; glioblastoma tumors; and other carcinoma tumors; non-small cell lung cancer; lung cancer; breast cancer; prostate cancer; and cancer of other organs. Further, disorders or conditions that may be treated using AMD-Exp 2, includes: disorders treated by inhibiting or promoting angiogenesis or by
5 inducing stasis of angiogenesis.

The active compounds may be administered in the form of a pharmaceutical composition formulated according to well known principles and incorporating the compound, preferably in unit dose form, in combination with a pharmaceutically acceptable diluent, carrier
10 or excipient. Such compositions may be in the form of solutions or suspensions for injection, or irrigation or be in capsule, tablet, dragee, or other solid composition or as a solution or suspension for oral administration or formulated into pessaries or suppositories or sustained release forms of any of the above for implantation. Suitable diluents, carriers, excipients and other components are well known. It may be desirable also to formulate a composition for
15 topical administration such as an ointment or cream. The compounds of the invention may be used, in the form of a composition or alone.

The pharmaceutical compositions according to the invention may be formulated in unit dosages determined in accordance with conventional pharmacological methods, suitably to provide active compounds in the dosage range in humans or animals of from 0.01 to 100 mg/kg
20 body weight per day, in a single dose or in a number of smaller doses. Preferred dosage ranges are 0.01 to 30 mg/kg body weight per day intravenous (iv) or intraperitoneal (ip). Other active compounds may be used in the compositions or such active compounds or supplemental therapy may be included in a course of treatment. The pharmaceutical compositions are useful for treatment of a patient comprising an effective therapeutic amount of the novel compound,
25 where said compound effectively binds to a chemokine receptor.

The present invention further contemplates the use of these compositions in the manufacture of a medicament for the treatment of HIV-or FIV-infected patients and/or the treatment of a disease by the regulation of endothelial cell function and/or the treatment of a disease relating to vascularization of the gastrointestinal tract; haematopoiesis; or cerebellar
30 development.

In a method for treating a patient infected with HIV or FIV, the pharmaceutical

composition is administered to said patient as a therapeutically effective amount of a pharmaceutical composition in a pharmaceutically acceptable carrier. In a method of treating a patient with a disease related to the regulation of endothelial cell function, the pharmaceutical composition is administered to said patient as a therapeutically effective amount of a pharmaceutical composition in a pharmaceutically acceptable carrier. The present invention further contemplates methods of treating a patient with a disease relating to vascularization of the gastrointestinal tract; haematopoiesis; or cerebellar development, by administering to said patient a therapeutically effective amount of a pharmaceutical composition in a pharmaceutically acceptable carrier.

10 The present invention further contemplates a method of treating a patient with a disease relating to basal leukocyte trafficking or the extravasation and tissue infiltration of leukocytes in response to inciting antigens, by administering to said patient a therapeutically effective amount of a pharmaceutical composition in a pharmaceutically acceptable carrier. The present method also contemplates treating a patient, by administering to said patient a therapeutically effective amount of a pharmaceutical composition in a pharmaceutically acceptable carrier, wherein said compound effectively binds to a chemokine receptor.

20 The present invention further contemplates pharmaceutical compositions and methods of use for the treatment of humans or animals for: renal allograft rejection; inflammatory disease; cancer; central nervous system developmental disease; HIV; vasculature development disease; haematopoiesis and other chemokine mediated diseases or disorders. This invention further provides for the treatment of diseases, which include, but are not limited to: arthritis; multiple sclerosis; dementia from HIV or FIV infection, Parkinson's disease, Alzheimer's disease and inflammatory diseases. The pharmaceutical compositions and methods of use of the present invention further provide for the treatment of cancers, that include, but are not limited to those associated with: solid tumors; lymphoma; metastatic tumors; glioblastoma tumors; and other carcinomas tumors. The pharmaceutical compositions of the present invention are useful for the treatment of cancers that include, but are not limited to: non-small cell lung cancer; lung cancer; breast cancer; prostate cancer; and cancer of other organs.

30 Other diseases or disorders that are contemplated to be treated with the pharmaceutical compositions of the present invention, include, but are not limited to: disorders treated by

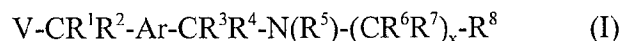
inhibiting or promoting angiogenesis or by inducing stasis of angiogenesis; developmental disorders mediated by chemokines.

The present invention further provides methods for the prevention of a disease or disorder in a patient by administering a therapeutically effective dosage of the pharmaceutical compositions of the present invention to a patient over a period of time sufficient to effectively prevent the disease or disorder.

CLAIMS

1. A macrocyclic compound of general formula I,

5



wherein V is a cyclic polyamine moiety having a total of 9 to 24 members and from 2 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

R¹ to R⁷ may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C₁₋₆ alkyl;

R⁸ is a heterocyclic group, a substituted aromatic group, or a mercaptan group;

Ar is an aromatic or heteroaromatic ring each optionally substituted at single or multiple positions with electron-donating or withdrawing groups;

x is 1 or 2;

and the acid addition salts and metal complexes thereof.

2. The macrocyclic compound of claim 1, wherein V is a 14 to 20 membered fused or unfused ring system.

3. A compound of claim 2, which is:

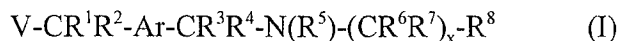
N-[4-(1,7-Diazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD-Exp 1).

4. A compound of claim 2, which is:

N-[7-(4,10-Diazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD-Exp 2).

30

5. A macrocyclic compound of general formula I,



wherein V is a cyclic polyamine moiety having a total of 9 to 24 members and from 3 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

R¹ to R⁷ may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C₁₋₆ alkyl;

R⁸ is a heterocyclic group, a substituted aromatic group, or a mercaptan group;

Ar is an aromatic or heteroaromatic ring each optionally substituted at single or multiple positions with electrons-donating or withdrawing groups;

x is 1 or 2;

and the acid addition salts and metal complexes thereof.

6. The macrocyclic compound of claim 5, wherein V is a 14 to 20 membered fused or unfused ring system.

7. The macrocyclic compound of claim 5, wherein V is a 4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl system or a 1,4,7-triazacyclotetra-decanyl or a 4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl system, or a 1,4,8,11-tetraazacyclotetradecanyl system or a derivative thereof.

8. A compound of claim 5, which is:

N-[4-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7049);

N-[7-(4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7050);

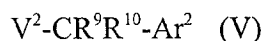
N-[7-(4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7051);

- N-[4-[4,7,10-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7058);
- N-[1-(1,4,7-Triazacyclotetra-decanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7059);
- 5 N-[4-[4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 7063);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-methyl)pyridine (AMD 3465);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-N-methyl-2-
- 10 (aminomethyl)pyridine (AMD 3538);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-amino-methyl)pyridine (AMD 3500);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-3-(amino-methyl)pyridine (AMD 3499);
- 15 N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-(2-amino-methyl-5-methyl)pyrazine (AMD 3498);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-ethyl)pyridine (AMD 3497);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-
- 20 methyl)thiophene (AMD 3516);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-(amino-ethyl)mercaptan (AMD 3530);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-2-amino-benzylamine (AMD 3517);
- 25 N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-amino-benzylamine (AMD 3544);
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-(amino-ethyl)imidazole (AMD 3543); or
- N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-benzylamine (AMD
- 30 3529);
- N-[3-(3,6,17-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-

phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD 8630);
N-[3-(3,6,17-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,3-
phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD8631);
N-[4-(4,7,17-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-
5 phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD7450);
N-[7-(4,7,17-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-
phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD7463);
N-[6-(3,6,9-Triazabicyclo[11.3.1]pentadeca-1(15),11, 13-trienyl)-1,3-
phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD7097); or
10 N-[7-(4,10,17-Triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-
phenylenebis(methylene)]-2-(aminomethyl)pyridine (AMD-Exp 3); or
the acid addition salts and metal complexes thereof.

9. A macrocyclic compound of general formula V,

15



wherein V^2 is a cyclic polyamine moiety having a total of 9 to 24 members and from 3 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic
20 ring;

R^9 and R^{10} may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C_{1-6} alkyl;

Ar^2 is an aromatic or heterocyclic ring each optionally substituted at single or multiple positions with electron-donating or withdrawing groups and/or aromatic and heterocyclic
25 groups and their alkyl derivatives thereof; and the acid addition salts and metal complexes.

10. The compound of claim 9, wherein V^2 is a cyclam or substituted cyclam system or a 1,4,8,11-tetraazacyclotetradecanyl system or a 4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl system.

30

11. The compound of claim 10, wherein Ar² is a heterocyclic or substituted heterocyclic.

12. The compound of claim 11, wherein the or heterocyclic substituents are
5 independently selected from the group consisting of: alkoxy; alkyl; halogen and hydrogen.

13. The compound of claim 12, which is:
1-[2,6-Dimethoxypyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 7032);
1-[2-Chloropyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 7048);
10 1-[2,6-Dimethylpyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 7060);
1-[2-Methylpyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 7061);
1-[2,6-Dichloropyrid-4-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 3451); or
1-[2-Chloropyrid-5-yl(methylene)]-1,4,8,11-tetraazacyclotetradecane (AMD 3454); or
the acid addition salts and metal complexes thereof.

15

14. The compound of claim 10, wherein Ar² is an aromatic or substituted aromatic ring.

15. The compound of claim 14, wherein the aromatic substituents are independently
20 selected from the group consisting of: alkoxy; alkyl; halogen; hydrogen; aromatic; heterocyclic
or substituted heterocyclic.

16. The compound of claim 15, which is:
N-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-purine (AMD 3472);
25 1-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-4-phenylpiperazine
(AMD3526); or
7-[4-methylphenyl (methylene)]-4,7,10,17-Tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene
(AMD 3484).

30 17. A pharmaceutical composition comprising an effective therapeutic amount of the
compound of any one of claims 1 to 16.

18. A composition according to claim 17, in unit dosage form.

19. The use of a composition of claim 17, in the manufacture of a medicament for
5 the treatment of HIV- or FIV-infected patients.

20. The use of a compound of claim 17, in the manufacture of a medicament for the
treatment of a disease by the regulation of endothelial cell function.

10 21. The use of a composition of claim 17, in the manufacture of a medicament for
the treatment of a disease relating to vascularization of the gastrointestinal tract;
haematopoiesis; or cerebellar development.

22. A method of treating a patient infected with HIV or FIV, by administering to
15 said patient a therapeutically effective amount of a pharmaceutical composition comprising the
composition of claim 17 in a pharmaceutically acceptable carrier.

23. A method of treating a patient with a disease related to the regulation of
endothelial cell function, by administering to said patient a therapeutically effective amount of a
20 pharmaceutical composition comprising the composition of claim 13 in a pharmaceutically
acceptable carrier.

24. A method of treating a patient with a disease relating to vascularization of the
gastrointestinal tract; haematopoiesis; or cerebellar development, by administering to said
25 patient a therapeutically effective amount of a pharmaceutical composition comprising the
composition of claim 17 in a pharmaceutically acceptable carrier.

25. A method of treating a patient with a disease relating to basal leukocyte
trafficking or the extravasation and tissue infiltration of leukocytes in response to inciting
30 antigens, by administering to said patient a therapeutically effective amount of a pharmaceutical
composition comprising the composition of claim 17 in a pharmaceutically acceptable carrier.

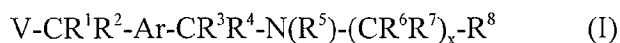
26. A pharmaceutical composition useful for treatment of a patient comprising an effective therapeutic amount of the compound of any one of claims 1 to 16, wherein said compound effectively binds to a chemokine receptor.

5

27. A method of treating a patient, by administering to said patient a therapeutically effective amount of a pharmaceutical composition comprising the composition of claim 17 in a pharmaceutically acceptable carrier, wherein said compound effectively binds to a chemokine receptor.

10

28. A pharmaceutical composition comprising a therapeutically effective amount of a compound of general formula I,



15

wherein V is a cyclic polyamine moiety having a total of 9 to 24 members and from 2 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

20 R^1 to R^7 may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C_{1-6} alkyl;

R^8 is a heterocyclic group, a substituted aromatic group, or a mercaptan group;

Ar is an aromatic or heteroaromatic ring each optionally substituted at single or multiple positions with electrons-donating or withdrawing groups;

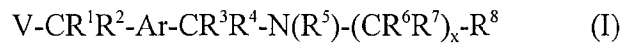
25 x is 1 or 2;

and the acid addition salts and metal complexes thereof, and

wherein said pharmaceutical composition is useful for the treatment of inflammatory disease; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; haematopoiesis and other chemokine mediated diseases or disorders.

30

29. A pharmaceutical composition comprising a therapeutically effective amount of a compound of general formula I,



5

wherein V is a cyclic polyamine moiety having a total of 9 to 24 members and from 3 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

10 R^1 to R^7 may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C_{1-6} alkyl;

R^8 is a heterocyclic group, a substituted aromatic group, or a mercaptan group;

Ar is an aromatic or heteroaromatic ring each optionally substituted at single or multiple positions with electrons-donating or withdrawing groups;

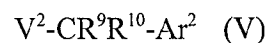
15 x is 1 or 2;

and the acid addition salts and metal complexes thereof, and

wherein said pharmaceutical composition is useful for the treatment of inflammatory disease; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; haematopoiesis and other chemokine mediated diseases or disorders.

20

30. A pharmaceutical composition comprising a therapeutically effective amount of a compound of general formula V,



25

wherein V^2 is a cyclic polyamine moiety having a total of 9 to 24 members and from 3 to 6 optionally substituted amine nitrogens spaced by two or more optionally substituted carbon atoms from each other, and which may optionally comprise a fused aromatic or heteroaromatic ring;

30 R^9 and R^{10} may be the same or different and are independently selected from hydrogen or straight, branched or cyclic C_{1-6} alkyl;

Ar² is an aromatic or heterocyclic ring each optionally substituted at single or multiple positions with electron-donating or withdrawing groups and/or aromatic and heterocyclic groups and their alkyl derivatives thereof; and the acid addition salts and metal complexes, and

wherein said pharmaceutical composition is useful for the treatment of inflammatory
5 disease; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; haematopoiesis and other chemokine mediated diseases or disorders.

31. A pharmaceutical composition comprising a therapeutically effective amount of a compound of: AMD 3100 ((1,1'-[1,4-phenylenebis(methylene)])bis-1,4,8,11-
10 tetraazacyclotetradecane), wherein said pharmaceutical composition is useful for the treatment of inflammatory disease; organ transplant rejection; cancer; central nervous system developmental disease; HIV; FIV; vasculature development disease; cardiogenesis developmental disease; haematopoiesis and other chemokine mediated diseases or disorders.

32. The pharmaceutical composition of claim 28, 29, 30 or 31, wherein said disease
15 is arthritis.

33. The pharmaceutical composition of claim 28, 29, 30 or 31, wherein said disease
is multiple sclerosis.

20

34. The pharmaceutical composition of claim 28, 29, 30 or 31, wherein said cancer is associated with: solid tumors; lymphoma; metastatic tumors; glioblastoma tumors; and other carcinoma tumors.

35. The pharmaceutical composition of claim 34, wherein said cancer is; non-small
25 cell lung cancer; lung cancer; breast cancer; prostate cancer; and cancer of other organs.

36. A pharmaceutical composition of claim 28, 29, 30 or 31, wherein said disorder is treated by inhibiting or promoting angiogenesis or by inducing stasis of angiogenesis.

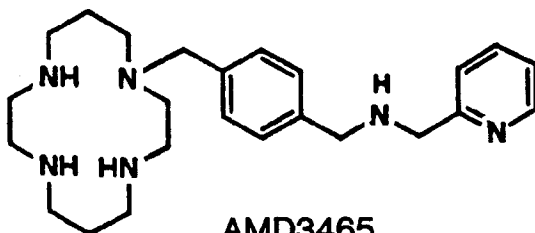
30

37. A method for the treatment of a patient comprising, administering a therapeutically effective dosage of the pharmaceutical composition of claim 28, 29, 30 or 31, to a patient with a disease or disorder that responds to said treatment over a period of time sufficient to effectively treat said disease or disorder.

5

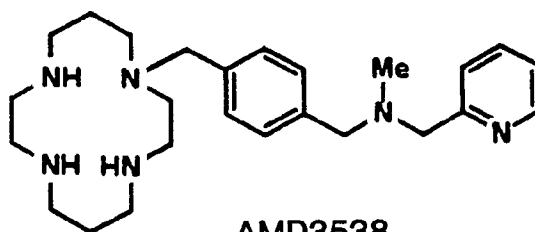
38. A method for the prevention of a disease or disorder in a patient comprising, administering a therapeutically effective dosage of the pharmaceutical composition of claim 28, 29, 30 or 31, to a patient over a period of time sufficient to effectively prevent said disease or disorder.

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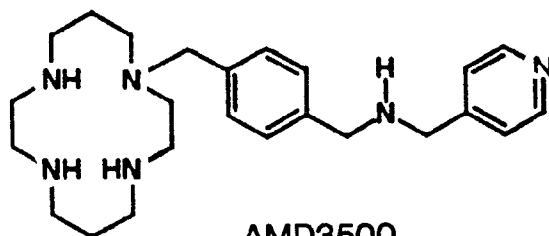
AMD3465

FIG. 1



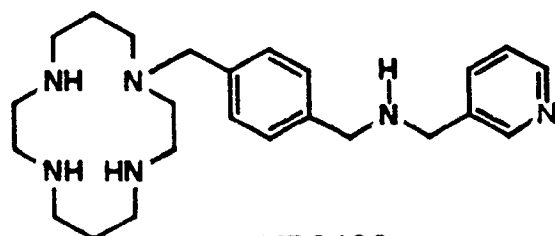
AMD3538

FIG. 2



AMD3500

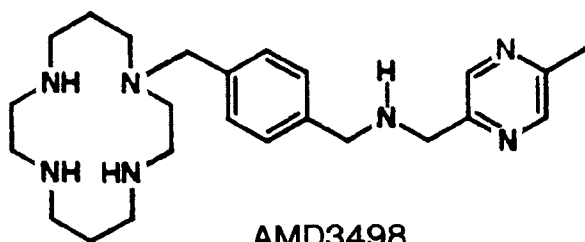
FIG. 3



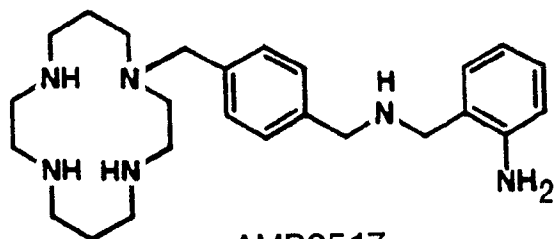
AMD3499

FIG. 4

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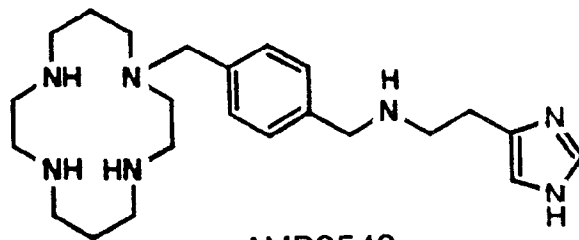
AMD3517

FIG. 9



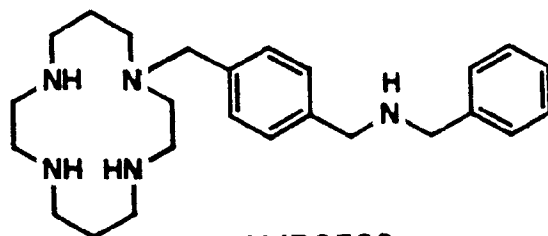
AMD3544

FIG. 10



AMD3543

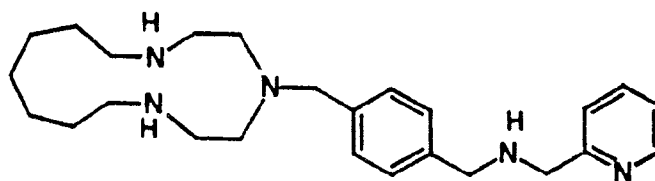
FIG. 11



AMD3529

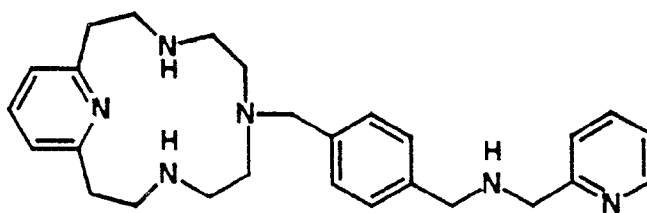
FIG. 12

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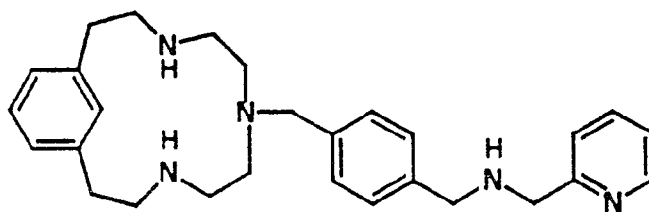
AMD7049

FIG. 13



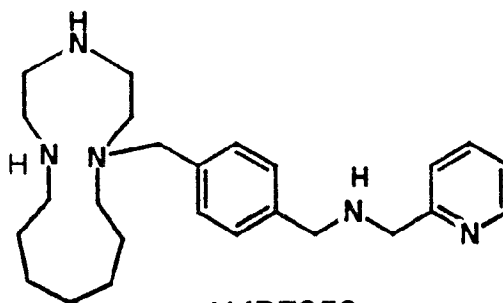
AMD7050

FIG. 14



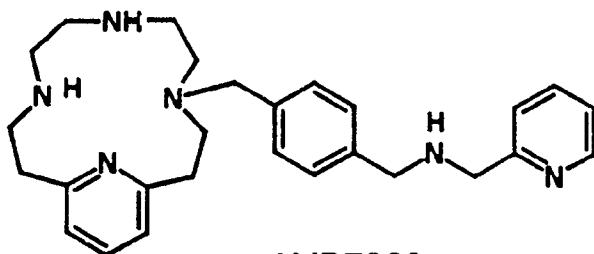
AMD7051

FIG. 15



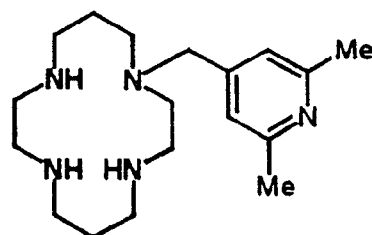
AMD7059

FIG. 16



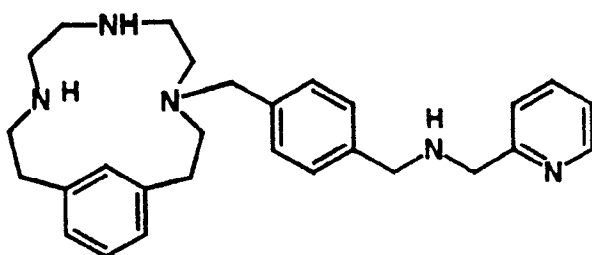
AMD7063

FIG. 17



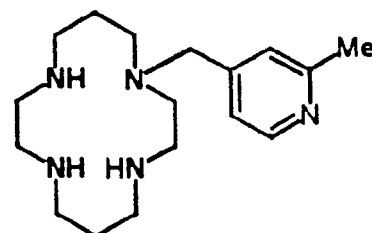
AMD7060

FIG. 21



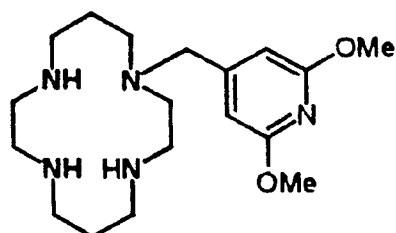
AMD7058

FIG. 18



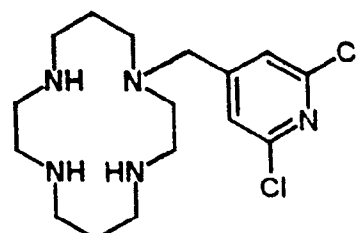
AMD7061

FIG. 22



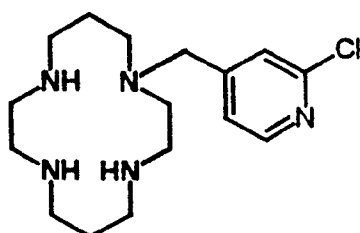
AMD7032

FIG. 19



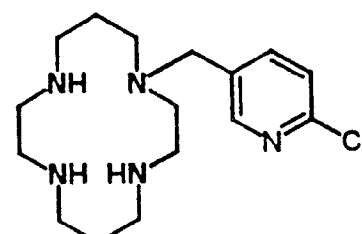
AMD3451

FIG. 23



AMD7048

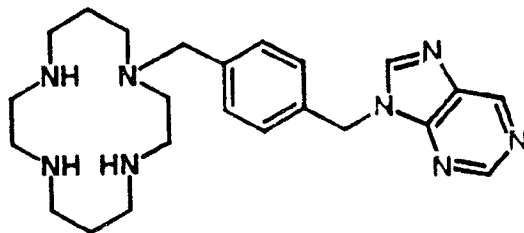
FIG. 20



AMD3454

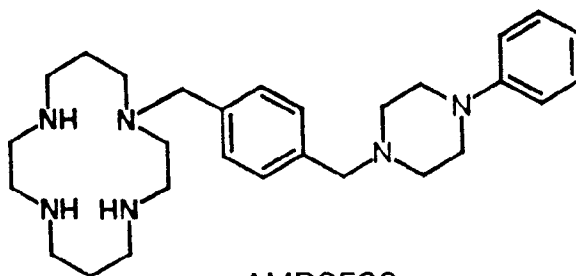
FIG. 24

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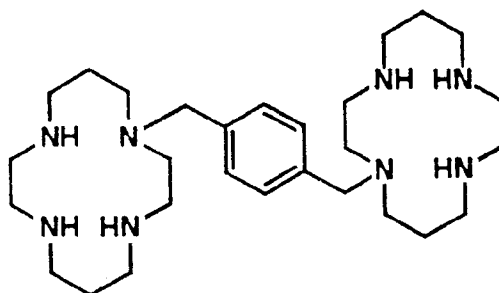
AMD3472

FIG. 25



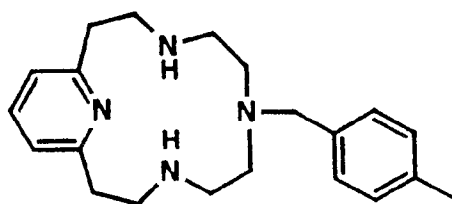
AMD3526

FIG. 26



AMD3100

FIG. 27



AMD3484

FIG. 28

SUBSTITUTE SHEET (RULE 26)

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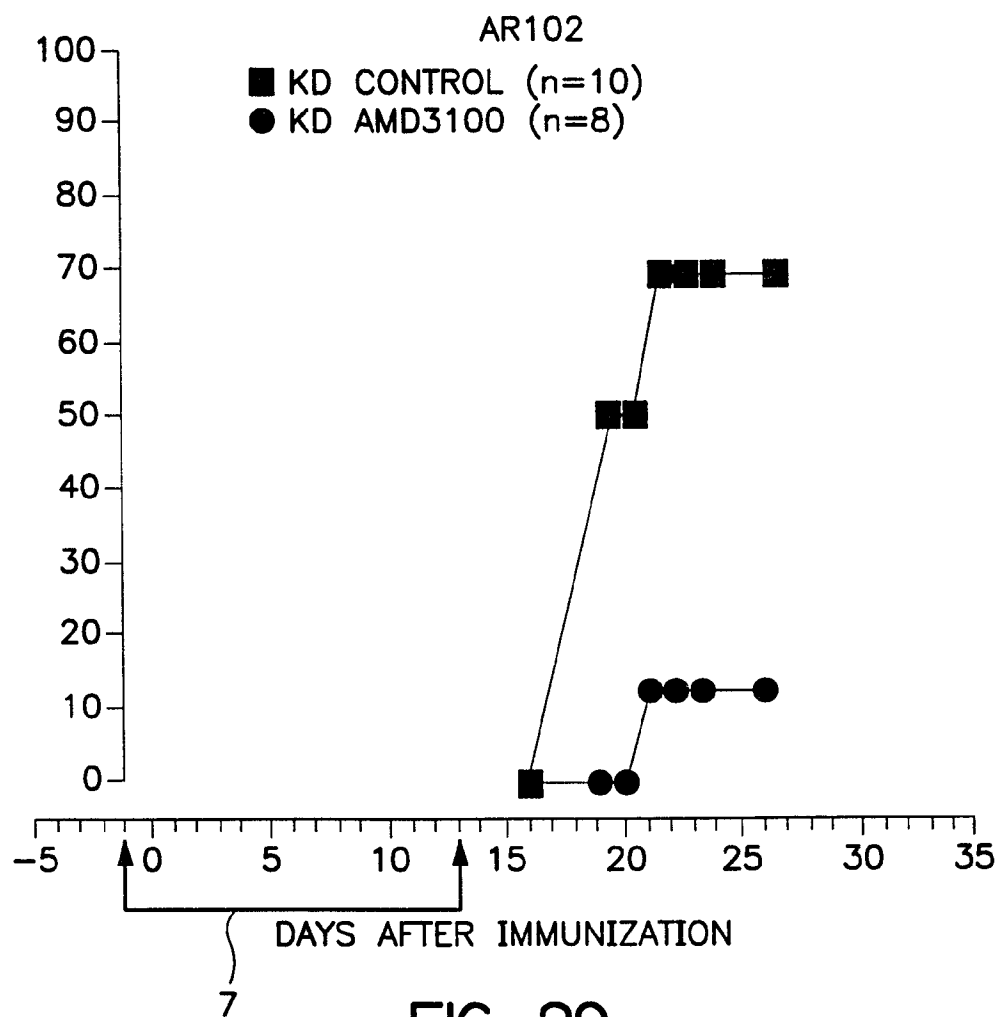


FIG. 29

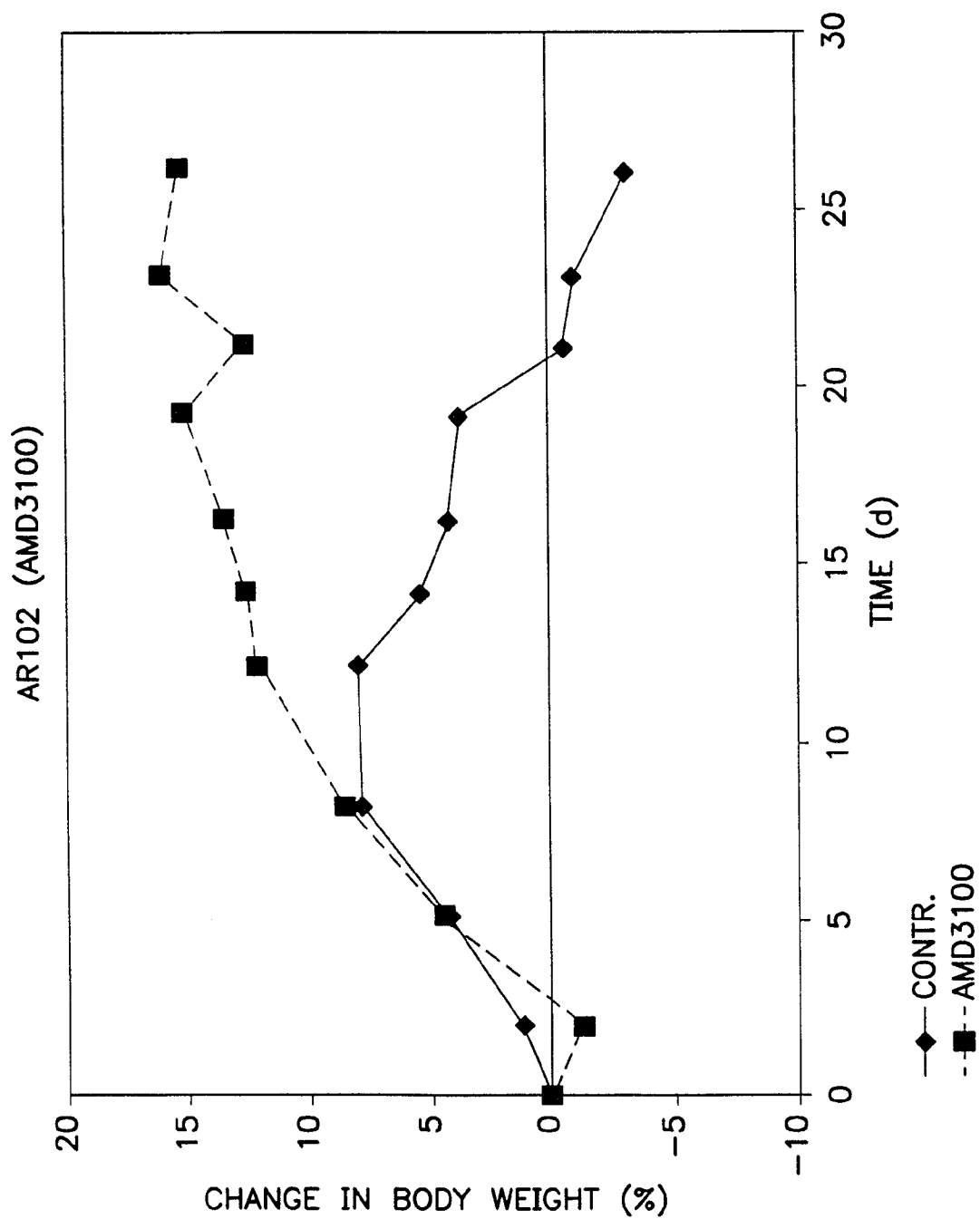
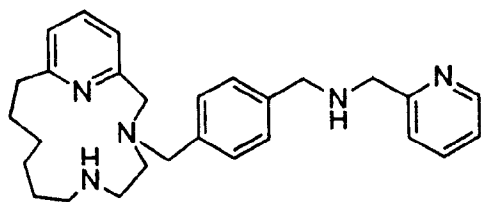
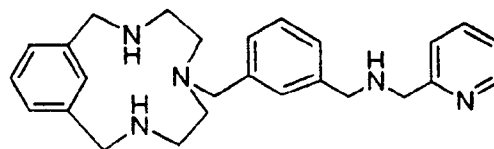


FIG. 30



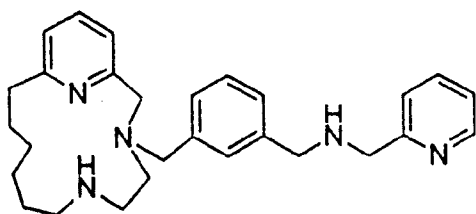
AMD8630

FIG. 31



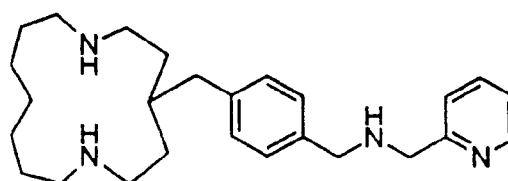
AMD7097

FIG. 32



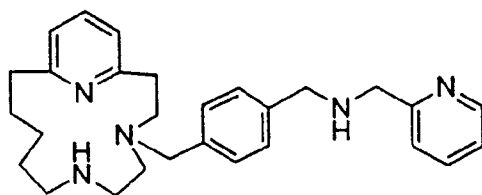
AMD8631

FIG. 33



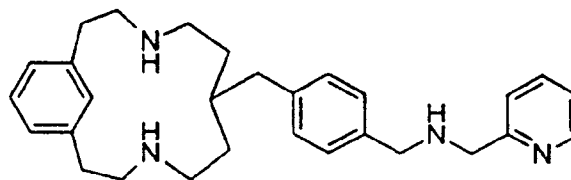
AMD-Exp 1

FIG. 34



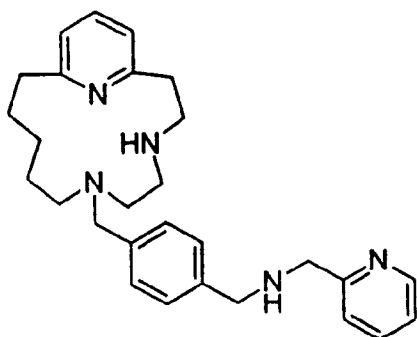
AMD7450

FIG. 35



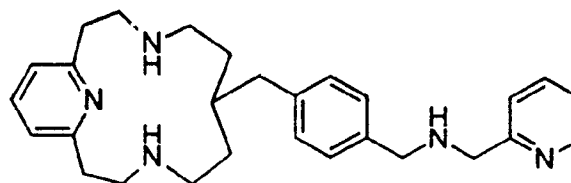
AMD-Exp 2

FIG. 36



AMD7463

FIG. 37



AMD-Exp 3

FIG. 38

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00619

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D257/02 C07D401/12 C07D409/12 C07D403/12 C07D471/06
C07D401/06 C07D473/00 A61K31/395 //(C07D471/06,257:00,
221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 747 368 A (JOHNSON MATTHEY PLC) 11 December 1996 (1996-12-11) the whole document & US 5 817 807 A cited in the application ---	1-38
Y	WO 93 12096 A (JOHNSON MATTHEY PLC) 24 June 1993 (1993-06-24) claims & US 5 583 131 A cited in the application ---	1-38
Y	WO 95 18808 A (JOHNSON MATTHEY PLC ;BRIDGER GARY JAMES (US); PADMANABHAN SREENIVA) 13 July 1995 (1995-07-13) claims & US 5 698 546 A cited in the application ---	1-38
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

14 October 1999

Date of mailing of the international search report

02/11/1999

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Chouly, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00619

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 16494 A (JOHNSON MATTHEY PLC) 1 October 1992 (1992-10-01) claims ---	1-38
A	BRIDGER G J ET AL: "SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF PHENYLENEBIS(METHYLENE)-LINKED BIS-TETRAAZAMACROCYCLES THAT INHIBIT HIV REPLICATION. EFFECTS OF MACROCYCLIC RING SIZE AND SUBSTITUENTS ON THE AROMATIC LINKER" JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 2, 20 January 1995 (1995-01-20), pages 366-378, XP000196379 ISSN: 0022-2623 the whole document ---	1-38
A	JOAO H C ET AL: "QUANTITATIVE STRUCTURAL ACTIVITY RELATIONSHIP STUDY OF BIS-TETRAAZACYCLIC COMPOUNDS. A NOVEL SERIES OF HIV-1 AND HIV-2 INHIBITORS" JOURNAL OF MEDICINAL CHEMISTRY., vol. 38, no. 19, 1995, pages 3865-3873, XP002118783 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US ISSN: 0022-2623 the whole document -----	1-38

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 99/ 00619

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 22-25, 27, 37-38
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 22-25, 27, 37-38
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 99/00619

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0747368 A	11-12-1996	AU 5245596 A	19-12-1996
		CA 2178272 A	07-12-1996
		JP 9012560 A	14-01-1997
		NO 962325 A	09-12-1996
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		US 5817807 A	06-10-1998
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		CZ 9401188 A	18-10-1995
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		HU 67544 A	28-04-1995
		IL 103984 A	30-10-1998
		JP 7501816 T	23-02-1995
		MX 9207316 A	01-06-1993
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